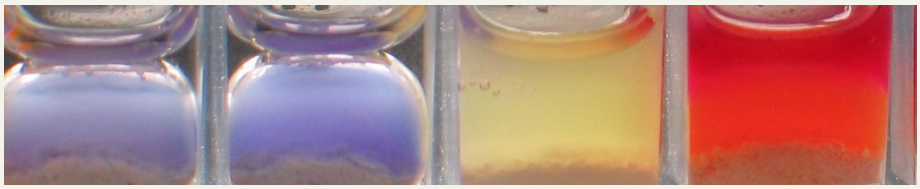


User's Guide

DIATABS™

DIAGNOSTIC TABLETS
FOR BACTERIAL IDENTIFICATION

7th Ed.
2007

TAASTRUPGAARDSVEJ 30 DK-2630 TAASTRUP DENMARK
Phone: +45 43 99 33 77 Telefax: +45 43 52 73 74 e-mail: info@rosco.dk

ROSCO
DIAGNOSTICA

USER's GUIDE DIATABS™

DIAGNOSTIC TABLETS FOR BACTERIAL IDENTIFICATION 7th Ed. 2007

Contents

1 Introduction 4
2 Bacterial identification using Diatabs 5
3 Diatabs in alphabetical order 9
3.1 ACETAMIDE HYDROLYSIS (ACM) 9
3.2 ALKALINE PHOSPHATASE (Alk P) 10
3.3 AMINOPEPTIDASES 12
3.3.1 GAMMA-GLUTAMYL AMINOPEPTIDASE (γ-GLU) 13
3.3.2 LEUCINE AMINOPEPTIDASE (LAP) 16
3.3.3 PROLINE AMINOPEPTIDASE (PRO) 18
3.3.4 PYRROLIDONYL AMINOPEPTIDASE (PYR) 20
3.3.5 TRYPSIN (BAA) (TRYP) 23
3.4 Anaerobes, Presumptive Identification with Oxgall (bile), Brilliant Green and Antibiotic Tablets 27
3.5 ARGININE DIHYDROLASE (ADH) 29
3.6 BACITRACIN LOW (BaL) 31
3.7 BACITRACIN 40 UNITS (BACIT) Neo-Sensitabs 32
3.8 BETA LACTAMASE (Acido) 33
3.9 Beta-lactamases (ESBL, AmpC, MBL) detection using Neo-Sensitabs and Diatabs 34
3.10 ESCULIN HYDROLYSIS (ESC) BILE ESCULIN (BE) 38
3.11 C-390 41
3.12 CITRATE (CIT) 42
3.13 CYCLOHEXIMIDE (CYC) 43
3.14 DEFEROXAMINE (DEFRX) 44
3.15 DOUBLE TEST Diatabs 46
3.15.1 LDC / INDOLE (LDC/IND) 47
3.15.2 ODC / INDOLE (ODC/IND) 48
3.15.3 PGUA / INDOLE (PGUA/IND) 50
3.15.4 UREASE / INDOLE (URE/IND) 51
3.15.5 UREASE / TDA (URE/TDA) 53
3.16 GENTAMICIN 250 µg (GN250), KANAMYCIN 500 µg (KA500), STREPTOMYCIN 500 µg (ST500) Neo-Sensitabs 54
3.17 FACTOR X, V, and X+V 56
3.18 FOSFOMYCIN 70 µg (FOSFO) Neo-Sensitabs 58
3.19 FURAZOLIDONE 50 µg (FURAZ) MUPIROCIN 10 µg (MUPIR) Neo-Sensitabs 60
3.20 GLYCOSIDASES 62
3.20.1 BETA-N-ACETYLGLUCOSAMINIDASE (ß-NAG) 64
3.20.2 ALPHA-FUCOSIDASE (α-FUC) 66
3.20.3 BETA-FUCOSIDASE (ß-FUC) 69
3.20.4 ALPHA-GALACTOSIDASE (α-GAL) 70
3.20.5 ONPG - Beta-Galactosidase (ONPG) 71

© Copyright Rosco Diagnostica A/S

3.20.6 ALPHA GLUCOSIDASE (α-GLU) BETA-GLUCOSIDASE (ß-GLU) 73
3.20.7 BETA-GLUCURONIDASE (PGUA) 75
3.20.8 ALPHA-MANNOSIDASE (α-MAN) 76
3.20.9 BETA-XYLOSIDASE (ß-XYL) 77
3.21 HIPPURATE HYDROLYSIS (HIP) 79
3.22 INDOXYL ACETATE (IAC) 82
3.23 LYSINE DECARBOXYLASE (LDC) ORNITHINE DECARBOXYLASE (ODC) 84
3.24 METRONIDAZOLE 5 µg (MTR.5) 86
3.25 METRONIDAZOLE 50 µg (MTR50) 87
3.26 NITRATE REDUCTION (NO_3) 88
3.27 NOVOBIOCIN 5 µg (NOVO5) Neo-Sensitabs 90
3.28 O/129 (Vibriostaticum) (O/129) 92
3.29 OPTOCHIN (OPT) OXGALL (OXG) 94
3.30 OXIDASE (OXI) 96
3.31 POLYMYXINS 150 µg (CO150) Neo-Sensitabs 97
3.32 PORPHYRIN (d-Ala) (ALA) 99
3.33 PS. AERUGINOSA SCREEN (PSAER) 102
3.34 PYRAZINAMIDASE (PZA) 103
3.35 S.P.S. (SPS) 105
3.36 SUGAR FERMENTATION Tests (SFT) 107
3.37 TDA or INDOLE (TDA or IND) 111
3.38 TELLUR (TEL) 113
3.39 TETRATHIONATE REDUCTASE (TTR) 114
3.40 TRIBUTYRIN (TRIB) 115
3.41 UREASE (URE) 117
3.42 VOGES-PROSKAUER (VP) 120
4 Reagents 123
5 Useful TABLES for bacterial identification / differentiation 124
6 Alphabetic INDEX of Abbreviations and Codes 128

© Copyright Rosco Diagnostica A/S

1 Introduction

DIATABS™ DIAGNOSTIC TABLETS for BACTERIAL IDENTIFICATION

The **DIATABS™**, Diagnostic Tablets (D.T.), developed by Rosco are identification tests made available as individual tablets, which allow the microbiologist a free choice of the most appropriate tests for identification. Most of the Diatabs™ are rapid tests (chromogenic enzymatic reactions, and modified conventional tests). The tablets may be used as single tests to show isolated microbial properties or as part of cost-effective systems of their own.

This User's Guide describes more than 80 different types of tests for identification of the clinically important groups of bacteria, and has been written by J.B. Casals on behalf of Rosco Diagnostica. The 7th Ed. 2007 of the DIATABS™ User's Guide contains updated text, tables and references, all necessary information when using Diatabs tablets for identification of bacteria and yeasts.
A new chapter have been added with information how beta-lactamases (ESBL, AmpC, MBL) may be detected by phenotypical profiles using Neo-Sensitabs and Diatabs. There are new tables including new described species, such as *Staphylococcus pseudintermedius*, *Streptococcus pseudopneumoniae* and new genus such as Aggregatibacter and Parabacteroides.

Finally, we would like to quote The Manual of Clinical Microbiology 8th Ed. 2003, page 893:
"Individually available tablets … (Rosco Diagnostic Tablets …) are much cheaper than commercial kits, they can be applied in a number of situations and allow flexibility in tailoring the set to best suit special needs".

The User's Guide is available at our website www.rosco.dk and updated information is continuously included.

ROSCO Diagnostica A/S is welcoming any feedback and questions on bacterial identification from users directly (info@rosco.dk) or through our representatives.

ROSCO DIAGNOSTICA A/S

© Copyright Rosco Diagnostica A/S

2 Bacterial identification using Diatabs

Rosco Item No.	Diatabs	Use	Chapter
55721	Acetamide Hydrolysis (25)	Non-Fermenters	3.1
52011	Adonitol (50)	Enterobacteriaceae/Staphylococci etc.	3.36
55921	Alkaline Phosphatase (25)	Staphylococci/Anaerobes/Gemella	3.2
50111	Alpha-Fucosidase (50)	Anaerobes/Streptococci	3.20.2
50211	Alpha-Galactosidase (50)	Non-fermenters/Streptococci/Anaerobes	3.20.4
50411	Alpha-Glucosidase (50)	Non-fermenters/Anaerobes/Gardnerella	3.20.6
50711	Alpha Mannosidase (50)	*Listeria* spp. /Arcanobacterium/Actinomyces	3.20.8
52121	l-Arabinose (25)	Enterobacteriaceae/Staphylococci etc.	3.36
56211	Arginine Dihydrolase (ADH) (50)	Staphylococci/Streptococci/Non-fermenters/ Vibrionaceae/Lactic bacteria (Vanco R)	3.5
40211	Bacitracin Low (50)	Group A-streptococci/Gardnerella	3.6
70812	Bacitracin 40 U Neo-Sensitabs (50)	Screening *Haemophilus* spp.	3.7
50021	Beta-N-Acetylglucosaminidase (25)	Anaerobes/Streptococci/Actinomyces	3.20.1
59921	Beta-Fucosidase (25)	Streptococcus anginosus group	3.20.3
50311	Beta-Galactosidase (ONPG) (50)	Neisseria/Enterobacteriaceae/Non-Fermenters/ Anaerobes/Actinobacillus/Pasteurella	3.20.5
50511	Beta-Glucosidase (50)	Staphylococci/Streptococci	3.20.6
50611	Beta-Glucuronidase (PGUA) (50)	*E.coli*/Enterobacteriaceae/Anaerobes/ Streptococci/Arcanobacterium	3.20.7
45521	Beta-Lactamase (25)	Haemophilus/Neisseria/Staphylococci	3.8
50811	Beta-Xylosidase (50)	Enterobacteriaceae/Capnocytophaga/ Acinetobacter	3.20.9
40421	Bile Esculin (25)	Enterococci, Lactic bacteria (Vanco R)	3.10
10041	Boronic Acid (25)	Detection of AmpC	3.9
40511	Brilliant Green (50)	Anaerobes	3.4
41611	C-390 40 µg (50)	*Pseudomonas aeruginosa*	3.11
	Cellobiose (25)	Enterobacteriaceae/Staphylococci etc.	3.36
56511	Citrate (50)	Enterobacteriaceae/Non-Fermenters	3.12
10031	Cloxacillin 500 µg (25)	Detection of AmpC	3.9
41811	Colistin 10 µg (50)	Anaerobes/Neisseria/Non-Fermenters	3.4
58921	Cycloheximide (50)	*Candida* spp.	3.13
59611	Deferoxamine 250 µg (50)	*Staph. epidermidis/Staph. hominis*, Non-Fermenters	3.14
	Dipicolinic Acid	Detection of MBL	3.9
	Dulcitol (25)	Enterobacteriaceae/Staphylococci etc.	3.36
56611	Esculin Hydrolysis (50)	Streptococci/Enterococci/Yersinia	3.10
42611	Factor V (50)	Haemophilus	3.17
42511	Factor X (50)	Haemophilus	3.17
42711	Factor X+V (50)	Haemophilus	3.17
74212	Fosfomycin Neo-Sensitabs (50)	Staphylococci, Corynebacteria	3.18
	Fructose (25)	Enterobacteriaceae/Staphylococci etc.	3.36
74412	Furazolidone Neo-Sensitabs (50)	Staphylococci/Micrococci/Enterococci/ Corynebact.	3.19

The numbers in brackets indicate the number of tablets per vial/cartridge.

© Copyright Rosco Diagnostica A/S

Rosco Item No.	Diatabs	Use	Chapter
	Galactose (25)	Enterobacteriaceae/Staphylococci etc.	3.36
46711	Gamma-Glutamyl Aminopeptidase (50)	Meningococci/Helicobacter	3.3.1
43012	Gentamicin 250 µg Neo-Sensitabs (50)	HLR enterococci	3.16
52611	Glucose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
56711	Hippurate Hydrolysis (50)	Campylobacter/Gardnerella/Streptococci/ Facklamia/Abiotrophia	3.21
59551	Indoxyl Acetate (15)	Campylobacter/Helicobacter	3.22
	Inositol (25)		3.36
52711	Inulin (50)	Enterobacteriaceae/Staphylococci etc.	3.36
43112	Kanamycin 500 µg Neo-Sensitabs (50)	Anaerobes/HLR enterococci	3.16
52811	Lactose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
58411	LDC/Indole (50)	Enterobacteriaceae/Salmonella ID	3.15.1
46811	Leucine Aminopeptidase (50)	Campylobacter/Corynebacteria/ CAT neg. Gram+cocci	3.3.2
56811	Lysine Decarboxylase (LDC) (50)	Enterobacteriaceae/Vibrionaceae/ Corynebacteria	3.23
52911	Maltose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
53011	Mannitol (50)	Enterobacteriaceae/Staphylococci etc.	3.36
53111	Mannose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
53211	Melibiose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
59711	Metronidazole 5 µg (50)	Anaerobes	3.24
43611	Metronidazole 50 µg (50)	Gardnerella	3.25
75712	Mupirocin 10 µg Neo-Sensitabs (50)	Staphylococci/Micrococci/Enterococci/ Corynebact.	3.19
43711	Nitrate Reduction (50)	Staphylococci/Non-Fermenters, Anaerobes	3.26
46312	Novobiocin 5 µg Neo-Sensitabs (50)	Staphylococci/Peptostrep./Pediococci	3.27
45411	O/129 (Vibriostaticum) 150 µg (50)	Vibrionaceae, Corynebacteria	3.28
59121	ODC/Indole (25)	Enterobacteriaceae/Citrobacter spp.	3.15.2
50311	ONPG (Beta Galactosidase) (50)	Neisseria/Enterobacteriaceae/Non-Fermenters/ Anaerobes/Actinobacillus/Pasteurella	3.20.5
44211	Optochin (50)	Pneumococci	3.29
57011	Ornithine Decarboxylase (ODC) (50)	*Staph. lugdunensis*/Enterobacteriaceae/ Haemophilus/Corynebacteria	3.23
44311	Oxgall (Bile) (50)	Anaerobes	3.29
45711	Oxidase (50)	Enterobacteriaceae/Non-Fermenters/Neisseria	3.30
59011	PGUA/Indole (50)	*E. coli*	3.15.3
77512	Polymyxins 150 µg Neo-Sensitabs (50)	*Staph. aureus*/Shewanella/Kingella	3.31
57321	Porphyrin (d-ALA) (25)	Haemophilus/Gram positive cocci	3.32
46911	Proline Aminopeptidase (50)	Neisseria/Anaerobes/Clostridium difficile	3.3.3
59311	Ps. aeruginosa Screen (50)	*Ps. aeruginosa*	3.33
59811	Pyrazinamidase (50)	Corynebacteria/*Yersinia enterocolitica*	3.34
47011	Pyrrolidonyl Aminopeptidase (50)	Group A-streptococci/Enterobacteriaceae/ Peptostreptococci/Staphylococci/ Lactic bacteria (Vanco R)	3.3.4

The numbers in brackets indicate the number of tablets per vial/cartridge.

© Copyright Rosco Diagnostica A/S

Rosco Item No.	Diatabs	Use	Chapter
53311	Raffinose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
53411	l-Rhamnose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
	Ribose (25)	Enterobacteriaceae/Staphylococci etc.	3.36
	Salicin (25)	Enterobacteriaceae/Staphylococci etc.	3.36
53711	Sorbitol (25)	Enterobacteriaceae/Staphylococci etc.	3.36
44611	S.P.S. (50)	Gardnerella/Peptostreptococci	3.35
44712	Streptomycin 500 µg Neo-Sensitabs (50)	HLR enterococci	3.16
53811	Sucrose (25)	Enterobacteriaceae/Staphylococci etc.	3.36
57811	TDA or Indole (50)	Enterobacteriaceae/Anaerobes/ Actinobacillus/Pasteurella	3.37
45011	Tellur (50)	Enterococcus faecalis	3.38
57421	Tetrathionate Reductase (25)	Enterobacteriaceae/Non-Fermenters	3.39
53911	Trehalose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
48821	Tributyrin (25)	*Moraxella catarrhalis*/Non-Fermenters/ Corynebacteria	3.40
47211	Trypsin (50)	Non-Fermenters/Anaerobes/ Capnocytophaga	3.3.5
57511	Urease (50)	Enterobacteriaceae/Staphylococci/ Anaerobes/Non-Fermenters	3.41
57611	Urease/Indole (50)	Enterobacteriaceae/Anaerobes/ Actinobacillus/Pasteurella	3.15.4
57911	Urease/TDA (50)	Enterobacteriaceae	3.15.5
79312	Vancomycin 5 µg Neo Sensitabs (50)	Anaerobes/Enterococci	3.4
57711	Voges-Proskauer (50)	Enterobacteriaceae/Streptococci/ Staphylococci	3.42
54021	d-Xylose (25)	Enterobacteriaceae/Staphylococci etc.	3.36

AMINOPEPTIDASES:

46711	Gamma-Glutamyl Aminopeptidase (50)	Meningococci/Helicobacter	3.3.1
46811	Leucine Aminopeptidase (50)	Campylobacter/Corynebacteria/ CAT neg Gram+cocci	3.3.2
46911	Proline Aminopeptidase (50)	Clostridium difficile/Neisseria/ Peptostreptococci	3.3.3
47011	Pyrrolidonyl Aminopeptidase (50)	Group A-streptococci/Enterobacteriaceae/ Peptostreptocci/Staphylococci/Enterococci/ Lactic bacteria (Vanco R)	3.3.4
47211	Trypsin (50)	Non-Fermenters/Porphyromonas/ Capnocytophaga	3.3.5

DOUBLE TEST TABLETS:

58411	LDC/Indole (50)	Enterobacteriaceae	3.15.1
59121	ODC/Indole (25)	Enterobacteriaceae/*Citrobacter* spp.	3.15.2
59011	PGUA/Indole (50)	*E. coli*	3.15.3
57611	Urease/Indole (50)	Enterobacteriaceae/Anaerobes/ Actinobacillus/Pasteurella	3.15.4
57911	Urease/TDA (50)	Enterobacteriaceae	3.15.5

The numbers in brackets indicate the number of tablets per vial/cartridge.

© Copyright Rosco Diagnostica A/S

Rosco Item No.	Diatabs	Use	Chapter
ESTERASES/LIPASES:			
59551	Indoxyl Acetate (15)	Campylobacter/Helicobacter	3.22
48821	Tributyrin (25)	Moraxella catarrhalis/Non-Fermenters/ Corynebacteria	3.40
GLYCOSIDASES:			
50021	Beta-N-Acetylglucosaminidase (25)	Anaerobes/Streptococci/Actinomyces	3.20.1
50111	Alpha-Fucosidase (50)	Streptococci/Prevotella/Porphyromonas	3.20.2
59921	Beta-Fucosidase (25)	Streptococcus anginosus group	3.20.3
50211	Alpha-Galactosidase (50)	Streptococci/Prevotella/Clostridia	3.20.4
50311	ONPG (Beta Galactosidase) (50)	Neisseria/Enterobacteriaceae/ Non-Fermenters/Anaerobes/ Actinobacillus/Pasteurella	3.20.5
50411	Alpha-Glucosidase (50)	Non-Fermenters/Gardnerella/Anaerobes	3.20.6
50511	Beta-Glucosidase (50)	Staphylococci/Streptococci	3.20.6
50611	Beta-Glucuronidase (PGUA) (50)	*E. coli*/Anaerobes/Streptococci/ Arcanobacterium	3.20.7
50711	Alpha-Mannosidase (50)	*Listeria* spp./Arcanobacterium/ Actinomyces	3.20.8
50811	Beta-Xylosidase (50)	Enterobacteriaceae/Capnocytophaga/ Acinetobacter/Propionibacteria.	3.20.9
SUGAR FERMENTATION TESTS:			
52011	Adonitol (50)	Enterobacteriaceae/Staphylococci etc.	3.36
52121	l-Arabinose (25)	Enterobacteriaceae/Staphylococci etc.	3.36
	Cellobiose (25)	Enterobacteriaceae/Staphylococci etc.	3.36
	Dulcitol (25)	Enterobacteriaceae/Staphylococci etc.	3.36
	Fructose (25)	Enterobacteriaceae/Staphylococci etc.	3.36
	Galactose (25)	Enterobacteriaceae/Staphylococci etc.	3.36
52611	Glucose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
	Inositol (25)	Enterobacteriaceae/Staphylococci etc.	3.36
52711	Inulin (50)	Enterobacteriaceae/Staphylococci etc.	3.36
52811	Lactose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
52911	Maltose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
53011	Mannitol (50)	Enterobacteriaceae/Staphylococci etc.	3.36
53111	Mannose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
53211	Melibiose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
53311	Raffinose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
53411	l-Rhamnose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
	Ribose (25)	Enterobacteriaceae/Staphylococci etc.	3.36
53621	Salicin (25)	Enterobacteriaceae/Staphylococci etc.	3.36
53711	Sorbitol (50)	Enterobacteriaceae/Staphylococci etc.	3.36
53811	Sucrose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
53911	Trehalose (50)	Enterobacteriaceae/Staphylococci etc.	3.36
54021	d-Xylose (25)	Enterobacteriaceae/Staphylococci etc.	3.36

The numbers in brackets indicate the number of tablets per vial/cartridge.

© Copyright Rosco Diagnostica A/S

3 Diatabs in alphabetical order

3.1 ACETAMIDE HYDROLYSIS (ACM)

REF No. 55721

Test for demonstration of the ability of bacterial strains to hydrolyse acetamide. Mainly used in differentiation of non-fermenting gram-negative rods.

Procedure
Prepare a dense bacterial suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one Acetmide Hydrolysis Diagnostic Tablet and close the tube.

Incubate at 35-37 °C for 18-24 hours - some positive reactions may be recorded already after 4-6 hours.

Reading of the tests
Positive reaction: **Red**
Negative reaction: Yellow, orange

Results

Acetamide hydrolysis is useful in the differentiation within the **fluorescent** group of Pseudomonas:

	ACM
Pseudomonas aeruginosa	+
Pseudomonas fluorescens	0^{+}
Pseudomonas putida	0

For the differentiation of *Comamonas acidovorans* (+) from *Comamonas testosteroni* (0). Most strains of *Burkholderia cepacia* are positive and most strains of *St. maltophilia* are negative.

Most strains of Alcaligenes (faecalis, denitrificans and *Achr. xylosoxidans*) are positive, while other non-fermenters are negative.

Non-fermenters

ACM positive	**ACM negative**
Ps. aeruginosa	*Ps. fluorescens*
Com. acidovorans	*Ps. putida*
Burkh. cepacia	*Com. testosteroni*
Alc. faecalis	*Sten. maltophilia*
Alc. denitrificans	
Achr. xylosoxidans	

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Acetamide hydrolysis (Acetamide)	*Ps. aeruginosa* ATCC 27853	*E. coli* ATCC 25922

References
1) Palleroni, N.J.: Pseudomonas in "Bergey's Manual of Systematic Bacteriology", Vol. 1, 141-199, 1984.

© Copyright Rosco Diagnostica A/S

3.2 ALKALINE PHOSPHATASE (Alk P)

REF No. 55921

Contain the chromogenic substrate: 4-nitrophenyl phosphatedi (2-amino-2-ethyl-1,3-propanediol) salt that in the presence of alkaline phosphatase releases free 4-nitrophenol (yellow colour).

Procedure
Prepare a dense bacterial suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one Alkaline Phosphatase Diagnostic Tablet and close the tube. Incubate at 35-37°C for a **maximum of 4 hours**.

Reading of the tests
Positive reaction: **Strong yellow**
Negative reaction: Colourless or slight yellow

Incubation longer than 4 hours may give a false positive reaction.

Results

1) Staphylococci
Most strains of *S. aureus* and *S. epidermidis* and *S. schleiferi* show a positive reaction, while most strains of *S. hominis*, *S. haemolyticus*, and *S. warneri* show a negative reaction.

	HCF	Alk P (4h)	PYR (1h)	ODC	URE	DEFRX	Poly
S. aureus	100	+	0	0	95	R (≤14 mm)	R (≤12 mm)
S. epidermidis	0	+	0	0^+	86	S (≥16 mm)	S
S. haemolyticus	0	0	100	0	0	R	S (≥14 mm)
S. hominis	0	0	0^+	0	+	S	S
S. lugdunensis	87	0^+	100	+	81	R	S
S. schleiferi	100	+	89	0	0	R	S
S. warneri	0	0	V	0	+	R	S

2) Differentiation of *Gemella* spp. and *Rothia mucilaginosa* (PYR +, LAP +)

	Alk P	SUC	SOR	NO_3	VP	BaciLow
Gemella bergeriae	0	0	0	0	0	R
Gemella haemolysans	+	V	0	0	V	R
Gemella morbillorum	0	+	0^+	·	0	R
Gemella sanguinis	+	+	+	0	V	R
Rothia mucilaginosa	0	+	0	+	+	S

Alk P = Alkaline Phosphatase D.T., PYR (1h) = Pyrrolidonyl Aminopeptidase D.T. (1h incubation), ODC = Ornithine Decarboxylase D.T., DEFRX = Deferoxamine D.T., URE = Urease D.T., Poly = Polymyxins 150 µg Neo-S, MAL = Maltose D.T., SUC = Sucrose D.T., SOR = Sorbitol D.T., HCF = Human clumping factor, NO_3 = Nitrate Reduction D.T., VP = Voges Proskauer D.T., BaciLow = Bacitracin Low D.T. (S ≥ 10 mm, R < 10 mm)

3) Useful also in the differentiation of non-fermenters, viridans streptococci, and anaerobes.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Alkaline Phosphatase (p-Nitrophenyl-Phosphate)	*E. coli* ATCC 25922	*S. haemolyticus* ATCC 29970

© Copyright Rosco Diagnostica A/S

References

1) Devriese L.A. et al: *Streptococcus hyointestinalis* sp. nov. from the gut of swine. Intl. J. Syst. Bacteriol. **38**, 440-1, 1988.
2) Lindsay J.A., Riley T.V.: Susceptibility to desferrioxamine: a new test for the identification of *Staphylococcus epidermidis*. J. Med. Microbiol. **35**, 45-48, 1991.
3) Collins M.D. et al: Description of *Gemella sanguinis* sp. nov. isolated from human clinical specimens. J. Clin. Microbiol. **36**, 3090-3, 1998.
4) Leung M.J.: Case of *Staph. schleiferi* endocarditis and a simple scheme to identify clumping factor positive staphylococci. J. Clin. Microbiol. **37**, 3353-6, 1999.

© Copyright Rosco Diagnostica A/S

3.3 AMINOPEPTIDASES

General description
Bacteria may be differentiated on their ability to enzymatically hydrolyze a series of aminopeptidase substrates. The procedure is based upon the enzymatic liberation of beta naphthylamine (beta-NA) from an l-aminoacid- beta-NA substrate. The liberated beta-NA is identified by its reaction with Aminopeptidase reagent producing a red colour in case of positive reactions.

Range
The actual range of aminopeptidases (substrates) comprises:

Gamma-Glutamyl Aminopeptidase	(γ-GLU)	(46711)
Leucine Aminopeptidase	(LAP)	(46811)
Proline Aminopeptidase	(PRO)	(46911)
Pyrrolidonyl Aminopeptidase	(PYR)	(47011)
Trypsin (BAA)	(TRYP)	(47211)

Procedure
Prepare a dense "milky" bacterial suspension (at least McFarland No. 4) from the strain to be tested in 0.25 ml saline in a tube. Add one tablet of aminopeptidase substrate and close the tube. Incubate at 35-37 °C for **4 hours**. In som cases, overnight incubation is required.
After incubation add 3 drops of Aminopeptidase reagent (92231) and read the colour reaction within 5 minutes.

Reading of the tests

	4 h	Overnight
Positive reaction:	**Red/orange**	**Red**
Negative reaction:	Yellow	Yellow/orange

The test may also be read by exposing the tube (no reagent added) to a Wood's lamp (at 360 nm). A blue fluorescence in the supernatant indicates a positive reaction.

References General
1) Peterson E.H., Hsu E.J.: Rapid detection of selected gram-negative bacteria by aminopeptidase profiles. J. Food Sci. **43**, 1853-1856, 1978.
2) Watson R.R.: Substrate specificities of aminopeptidases: a speci fic method for microbial differentiation. Methods Microbiol. **9**, 1-4, 1976.
3) Euzeby J.P.: Activité peptidasique vis a vis des aminoacyl-beta-naphtilamides de quelques especies du genre Bartonella. Dictionaire de Bacteriologie Veterinaire, Sept. 1999.

© Copyright Rosco Diagnostica A/S

3.3.1 GAMMA-GLUTAMYL AMINOPEPTIDASE (γ-GLU)

REF No. 46711

The test is based on enzymatic release of beta-naphthylamine from the gamma-glutamyl-beta-naphthylamide substrate. Beta-naphthylamine is detected by its reaction with Aminopeptidase reagent giving a red colour.

Procedure
Prepare a dense bacterial suspension with a turbidity of at least McFarland No. 4 of the strain to be tested in 0.25 ml saline in a tube. Add one Gamma-glutamyl aminopeptidase tablet and close the tube. Incubate at 35-37 °C for **4 hours**. After incubation add 3 drops Aminopeptidase reagent (92231) and read the colour reaction within 5 minutes.
See also Aminopeptidase, general description, page 12.

Reading of the tests (4h)
Positive reaction: **Red/orange**
Negative reaction: Yellow

Results

1) Neisseria/Moraxella (HTM + (V), OXI +, CAT +^{0})

	γ-GLU	NA35	SUP	TRIB	Remarks
N. menigitidis	+	0^+	V	0	
N. gonorrhoeae	0	0	+	0	Co 10 R
N. lactamica	0	$+^0$	V	0	ONPG +
N. cinerea	0	+	0	0	Co 10 S
N. polysaccharea	0	+	0	0	Co 10 R
M. catarrhalis	0	+	+	+	NO_3 +
Kingella denitrificans	0	·	0	0	Co 10 R, NO_3 +, CAT 0

Most *N. gonorrhoeae* are PRO (proline aminopeptidase) positive, but approx. 5 % are negative (5).

2) Acinetobacter

	γ-GLU	β-XYL
A. baumanii/calcoaceticus	$+^0$	+
A. lwoffi	0	0

3) Helicobacter

	γ-GLU	IAC
Helicobacter pylori	+	0
Helicobacter cinaedi	0	0wk
Helicobacter fennelliae	0	+

γ–GLU = Gamma-Glutamyl Aminopeptidase D.T., NA35 = Growth in nutrient agar at 35 °C, SUP = Superoxol (30 % H_2O_2) hydrogen peroxide, TRIB = Tributyrin D.T., HTM = Growth on Modified Thayer Martin medium, OXI = Oxidase D.T., CAT = Catalase, β-XYL = Beta-Xylosidase D.T., IAC = Indoxyl Acetate D.T., Co 10 = Colistin 10 µg (S≥10 mm).

© Copyright Rosco Diagnostica A/S

4a) Identification of clinically most common *Nocardia* spp. (3). 4 hours' tests except URE

	PYR	γ-GLU	α-GLU	α-MAN	URE	45 °C	Remarks
N. abscessus *	0	+	+	0	+	0	Imip S/R
N. brasiliensis	0	+	+	+	+	0	Imip R
N. cyriacigeorgica *	0	+	+	0	0	+	AMC I/R
N. farcinica	+	+	+	0	$+^{0}$	+	AMC S, Tobra R, Ery R
N. nova	+	0	+	0	V	0^{+}	Tobra I/R, AMC I/R, Ery S
N. paucivorans	0	+	0	0	0	+	

*) Members of the *N. asteroides* complex.

PYR = Pyrrolidonyl Aminopeptidase D.T., γ-GLU = Gamma-Glutamyl-Aminopeptidase D.T., α-GLU = Alpha-Glucosidase D.T., α-MAN = Alpha-Mannosidase D.T., URE = Urease D.T., 45 °C = growth at 45 °C.

4b) Identification of *Nocardia* spp. by antibiogram (6)

	AMOX	AMC	CEFTR	IMIP	CLARI	TOBRA
N. abscessus	S	S	S	V	V	S
N. brasiliensis	R^{S}	S	V	R	R	S
N. cyriacigeorgica	V	I/R	S	S	R	S
N. farcinica	R	S	R	S	R	R
N. nova	S	I/R	V	S	S	V

AMOX = Amoxycillin Neo-S, AMC = Amoxycillin+Clavulanate Neo-S, CEFTR = Ceftriaxone Neo-S, IMIP = Imipenem Neo-S, CLARI = Clarithromycin Neo-S, Tobra = Tobramycin Neo-S, Ery = Erythromycin Neo-S.

5) Salmonella and Shigella (4) serotypes

	γ-GLU	Remarks
S. typhimurium	80 %	
S. enteritidis	+	
S. blegdam	+	
S. berta	+	
S. gallinarum/pullorum	+	
S. typhi	+	
S. arizonae	0	PGUA 0
S. diarizonae	+	PGUA +
S. dublin	0	
S. naestved	0	
S. kiel	0	
Shigella dysenteriae		
- serotypes 3 to 9	+	
- serotypes 1, 2 and 10	0	
Shigella sonnei	0	
Salmonella serotype III a	0	
Salmonella serotype III b	+	

γ-GLU = Gamma-Glutamyl-Aminopeptidase D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Gamma-Glutamyl Aminopeptidase (Gamma-Glutamyl-β-Naphthylamide)	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922

© Copyright Rosco Diagnostica A/S

References (γ-GLU)

1) Mc Nulty C.A.M et al: Rapid identification of *Campylobacter Pylori* by preformed enzymes. J. Clin. Microbiol. **25**, 1683-6, 1987.
2) Nebreda T. et al: Urethritis caused by *Neiss. meningitidis* serogroup C. Clin. Microbiol. Infect. **5**, 57-8, 1999.
3) Wauters G et al: Distribution of Nocardia species in clinical samples and their routine rapid identification in the laboratory. J. Clin. Microbiol. **43**, 2624-8, 2005.
4) Giammanco G. et al: Interet taxonomique de la recherche de la γ-glutamyltransferase chez les Enterobacteriaceae. Ann Microbiol. (Inst. Pasteur) **131A**, 181-7, 1980.
5) Blackmore T. et al: Characterization of prolyl-iminopeptidase-deficient N. gonorrhoeae. J. Clin Microbiol., **43**, 4189-90, 2005.
6) Glupczynski Y. et al: Determination of antimicrobial susceptibility patterns of Nocardia spp. from clinical specimens by E-test. Clin Microbiol. Infect. **12**, 905-912, 2006.

© Copyright Rosco Diagnostica A/S

3.3.2 LEUCINE AMINOPEPTIDASE (LAP)

REF No. 46811

The test is based on enzymatic release of beta-naphthylamine from the 1-leucine-beta-naphtahylamid substrate. Beta-naphthylamine is detected by its reaction with Aminopeptidase reagent giving a red colour.

Procedure
Prepare a dense bacterial suspension with a turbidity of at least McFarland No. 4 of the strain to be tested in 0.25 ml saline in a tube. Add one Leucine aminopeptidase tablet and close the tube. Incubate at 35-37 °C for **4 hours**.
After incubation add 3 drops of Aminopeptidase Reagent (92231) and read the colour reaction within 5 minutes.
See also Aminopeptidase, general description, page 12.

Reading of the tests (4h)
Positive reaction: **Red/orange**
Negative reaction: Yellow

Results

1) Catalase negative gram-positive cocci

	LAP	PYR	Van5	ADH	BE	Remarks
Streptococcus spp.	+	0	S	V	10	
Enterococcus spp.	+	+	S^R	$+^0$	99	
Aerococcus viridans	0	+	S	0	60	
Aerococcus urinae	+	0	S	0	0	PGUA +
Leuconostoc spp.	0wk	0	R	0	90	
Pediococcus spp.	+	0	R	$+^0$	+	
Dolosigranulum pigrum	+	+	S	V	0	

LAP = Leucine Aminopeptidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., Van5 = Vancomycin 5 µg Neo-S (S ≥15 mm, R ≤13 mm), ADH = Arginine Dihydrolase D.T., BE = Bile Esculin D.T., PGUA = Beta-Glucuronidase D.T.

2) Corynebacteria nonlipophilic fermentative (most common)

Most strains are: CAT +, MOT 0, Fosfo R, Mupi R, PRO $+^0$, COL R, NALI R.

	PZA	LAP	URE	NO_3	MAL	O/129	Remarks
C. amycolatum *	+	0	V	$+^0$	80	R	dry
C. argentoratense	+	82	0	0	0	S	CAMP 0
C. coylae	+	·	0	0	0	R	CAMP +
C. diphtheriae	0	V	0	$+^0$	+	S	
C. glucuronolyticum *	+	+	67	V	26	S	PGUA+, CAMP +
C. kutscheri	$+^0$	+	+	$+^0$	+	·	PYR +
C. minutissinum	+	+	0	0	+	S	NAG $+^0$
C. pseudotuberculosis	0	0	+	V	+	R	CAMP rev, α-GLU 0^+
C. renale group	$+^0$	0	+	0	0	·	PGUA +
C. striatum *	+	82	0	+	0	S	creamy
C. ulcerans	0	62	+	0	+	V	CAMP rev, α-GLU +
C. xerosis	+	88	0	60	+	S	dry yellowish, α-GLU $+^0$

* Resistant or multidrugresistant

© Copyright Rosco Diagnostica A/S

3) Corynebacteria nonlipophilic nonfermentative

Most strains are: CAT +, MOT 0, Fosfo R, Mupi R, PRO $+^0$.

	LAP	NO_3	DNase	Colonies	Remarks
C. afermentans (ANF-1)	0	0	0	smooth	
C. auris	+	0	0	dry	
Turicella otididis (ANF-1 like)	+	0	+	creamy	
C. propinquum	60	+			
C. pseudodiphtericum	$+^0$	+			URE +

PZA = Pyrazinamidase D.T., LAP = Leucine Aminopeptidase D.T., NO_3 = Nitrate Reduction D.T., MAL = Maltose D.T., O/129=O/129 150 µg D.T. (S ≥ 16 mm, R < 16 mm), NAG = Beta-N-acetylglucosominidase D.T., DNase, URE = Urease D.T., CAT = Catalase, MOT = motility, Fosfo = Fosfomycin Neo-S (R = no zone), Mupi = Mupirocin Neo-S (R = no zone).

4) Globicatella and Aerococcus (3)

	Gram stain	PYR	LAP	Inulin
G. sanguinis	pairs/chains	75	0	93
A. viridans	clusters/tetrads	100	0	7
Enteroc. avium	short chains	95	89	7
Strept. uberis	short chains	100	100	100

PYR = Pyrrolidonyl Aminopeptidase D.T., LAP = Leucine Aminopeptidase D.T., Inulin D.T.

5) Aerococci (4) (Vancomycin S, CAT neg. cocci, dividing in 2 planes)

	PYR	LAP	VP	MAL	SUC
Aer. viridans	+	0	0^+	V	+
Aer. urinae	0	+	0	0	+
Aer. sanguinicola	+	+	0	+	+
Aer. christensenii	0	+	+	0	0
Aer. urinaehominis	0	0	0	+	+

PYR = Pyrrolidonyl Aminopeptidase D.T., LAP = Leucine Aminopeptidase D.T., VP = Voges-Proskauer D.T., MAL = Maltose D.T., SUC = Sucrose D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Leucine Aminopeptidase (L-Leucine-β-naphthylamide-HCL)	*S. bovis* ATCC 15351	*Aerococcus viridans* ATCC 700406

References (LAP)

1) Devriese L.A. et al: Identification of Enterococcus species isolated from foods of animal origin. Intl. J. Food Microbiol. **26**, 187-97, 1995.
2) Renaud F.N.R. et al: Identification of *Turicella otitidis* isolated from a patient with otorrhea associated with surgery: differentation from *Coryneb. afermentans* and *Coryneb. auris*. J. Clin. Microbiol. **34**, 2625-7, 1996.
3) Lynn Shewmaker P. et al: DNA relatedness, phenotypic characteristics and antimicrobial susceptibilities of *Globicatella sanguinis* strains. J. Clin. Microbiol. **39**, 4052-7, 2001.
4) Facklam R. et al: Phenotypic description and antimicrobial susceptibilities of *Aerococcus sanguinicola* isolates from human clinical samples. J. Clin. Microbiol. **41**, 2587-92, 2003.
5) Christensen J.J. et al: Aerococcus urinae: polyphasic characterization of the species. APMIS, **113**, 517-525, 2005.

© Copyright Rosco Diagnostica A/S

3.3.3 PROLINE AMINOPEPTIDASE (PRO)

REF No. 46911

The test is based on enzymatic release of beta-naphthylamine from the l-proline-beta-naphthylamide substrate. Beta-naphthylamine is detected by its reaction with Aminopeptidase reagent giving a red colour.

Procedure
Prepare a dense bacterial suspension with a turbidity of at least McFarland No. 4 of the strain to be tested in 0.25 ml saline in a tube. Add one Proline aminopeptidase tablet and close the tube. Incubate at 35-37 °C for **4 hours**.
After incubation add 3 drops of Aminopeptidase Reagent (92231) and read the colour reaction within 5 minutes.
See also Aminopeptidase, general description, page 12.

Reading of the tests (4h)
Positive reaction: **Red/orange**
Negative reaction: Yellow

Results

1a) Identification of *Clostridium difficile*

Most clostridia are: Kana 500 S^R, Vanco 5 S, Col R, CAT 0.

	PRO	CCFA growth	Remarks
C. difficile	+	+	ONPG 0, PYR 0, Alk P 0, Amox S, Merop S, Imipenem I/R
C. inocuum *	0	+	Vanco I/R, Teico S (van B)
C. perfringens	0	.	ONPG +, PYR +, Alk P +
C. ramosum	0	.	
C. sordelli	+	0	
C. bifermentans	+	0	
C. septicum	0	.	

PRO = Proline Aminopeptidase D.T., CCFA growth = Growth on CCFA medium, ONPG = ONPG Beta-Galactosidase D.T., Alk P = Alkaline Phosphatase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., Amox = Amoxycillin Neo-S, Merop = Meropenem Neo-S, Imipenem = Imipenem Neo-S, Vanco = Vancomycin Neo-S, Teico = Teicoplanin Neo-S.

* *C. inocuum* shows intrinsic low level resistance to vancomycin (MIC 8-16 µg/ml) with Van 5 zones < 18 mm.

1b) Rapid ID of common lecithinase positive *Clostridium* spp.

	IND	URE	PRO	NAG	Remarks
C. novyi type A	0^+	0	0	0	swarm
C. perfringens	0	0	0	+	PYR +
C. bifermentans	+	0	+	V	
C. sordelli	+	+	+	0	

1c) Rapid ID of swarming clostridia

	IND	ESC	PRO	LIP	Remarks
C. novyi type A	0^+	0	0	+	Strong beta haem.
C. septicum	0	+	0	0	
C. sporogenes	0	+	+	+	PYR $+^0$, NAG 0
C. tetani	$+^0$	0	0	0	

URE = Urease D.T., ESC = Esculin Hydrolysis D.T., LIP = Lipase.

© Copyright Rosco Diagnostica A/S

1d) Differentiation among Clostridia producing large cytotoxins

	PRO	IND	URE	LEC	Swarm
C. difficile	+	0	0	0	0
C. sordelli	+	+	+	+	0
C. novyi A	0	0^+	0	+	+

IND = Indole D.T., URE = Urease D.T., LEC = Lecithinase.

2) Differentiation of Peptostreptococci and similar (most current clinical isolates)

	PRO	PYR	GLU	α-GLU	IND	SPS	Alk P	Remarks
P. anaerobius	+	0	+	+	0	S (≥12 mm)	0	
P. asaccharolyticus	0	0	0	0	$+^0$	R	+	
M. micros	$+^0$	+	0	0	0	R	+	
F. magna	0	+	0	0	0	R	V	
P. stomatis	0	0	+	+	0	S (≥15 mm)	0	
A. vaginalis	0	0	+	V	0	R	V	LAP +
P. harei	0	0	0	0	0	R	0	

PRO = Proline Aminopeptidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., α-GLU = Alpha-Glucosidase D.T., IND = Indole D.T., SPS = SPS D.T., GLU = Glucose D.T., Alk P = Alkaline Phosphatase D.T.

3) Identification of *Candida albicans* (4 hours incubation) (5)

	PRO	NAG	α-GLU (2h)	42 °C
Candida albicans	+	$+^0$	+	+
C. dublinensis	+	$+^0$	0	0
Candida spp. (A)	+	0	-	-
Candida spp. (B)	0	0	-	-

where (A) comprises: *C. guilliermondii*, *C. lipolytica*, *C. lusitaniae*, *C. norvegensis*, *C. parapsilosis*, T*or. candida.*
where (B) comprises: *C. glabrata*, *C. kruseii*, *C. pseudotropicalis*, *C. rugosa* (NAG 0^+), *C. tropicalis* (NAG 0^+).

PRO = Proline Aminopeptidase D.T., NAG = Beta-N-Acetylglucosaminidase D.T., α-GLU(2h) = Alpha-Glucosidase D.T. (2 hours' incubation), 42 °C = Growth at 42 ° in Sabouraud Glucose Agar.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Proline Aminopeptidase (L-proline ß-Naphthylamide-HCl)	*P. aeruginosa* ATCC 27853	*Cl. perfringens* ATCC 12917

References (PRO)

1) Garcia A, Garcia T, Pérez J.L.: Proline aminopeptidase test for rapid screening of Clost. difficile. J. Clin. Microbiol. **35,** 3007, 1997.
2) Fedorko D.F. et al: Use of cycloserine-cefoxitin-fructose-agar (CCFA) and l-proline aminopeptidase in the rapid identification of Clostridium difficile. J. Clin. Microbiol. **35**, 1258-9, 1997.
3) Bourgault A.M. et al: Should all stool specimens be routinely tested for Clost. difficile. Clin. Microbiol. Infect. **5**, 219-22, 1999.
4) Murdoch D.A.: Gram-positive anaerobic cocci. Clin. Microbiol. Reviews. **11**, 81-120, 1998.
5) Niimi K. et al: Distinguishing *Candida* species by ß-N-acetylhexosaminidase activity. J. Clin. Microbiol. **39**, 2089-97, 2001.
6) Yuli Song et al: Development of a flow chart for identification of gram-positive anaerobic cocci in the clinical laboratory. J. Clin. Microbiol., **45**, 512-516, 2007.

© Copyright Rosco Diagnostica A/S

3.3.4 PYRROLIDONYL AMINOPEPTIDASE (PYR)

REF No. 47011

Some bacteria may be differentiated by their ability to enzymatically hydrolize an 1-pyrrolidonyl-beta-naphthylamide substrate. The liberated beta-naphthylamine is identified by reaction with Aminopeptidase reagent producing a red colour in case of positive reactions.

Procedure
Prepare a dense "milky" suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one PYR Diagnostic Tablet, close the tube and incubate at 35-37 °C for **4 hours** or **up to 18-24 hours**. In special cases an incubation period of 1 or 2 hours is used.

After incubation **add 3 drops of Aminopeptidase Reagent** (92231) and read the colour reaction within 5 minutes.

Reading of the test (4h)
Positive reaction: **Red/orange**
Negative reaction: Yellow

Results

1) Streptococci (2 hours incubation)

	PYR (2h)
S. pyogenes (haem A)	+
Enterococci	+
Other streptococci	0

2a) Most common human staphylococci

	PYR (1h)	ODC	Alk P (4h)	POLY	DEFRX
S. aureus	0	0	+	R (≤12 mm)	R (≤14 mm)
S. epidermidis	0	0^+	+	V	S (≥16 mm)
S. haemolyticus	+	0	0	S (≥14 mm)	R
S. hominis	0^+	0	0	S	S
S. lugdunensis	+	+	0^+	S	R
S. schleiferi	$+^0$	0	+	S	R

2b) Coagulase positive staphylococci

	PYR (1h)	VP (4h)	Poly	Pigment
S. aureus	0wk	+	R (≤12 mm)	+
S. intermedius	+	0wk	S (≥14 mm)	0
S. pseudintermedius	+	+	S (≥14 mm)	0

2c) Staphylococci (18-24h)

	PYR (18-24h)
S. aureus	+
S. epidermidis	0

PYR = Pyrrolidonyl Aminopeptidase D.T., ODC = Ornithine Decarboxylase D.T., VP = Voges-Proskauer D.T., Poly = Polymyxins 150 µg Neo-Sensitabs (S ≥ 14 mm, R ≤ 12 mm), Alk P = Alkaline Phospotase D.T., DEFRX = Deferoxamine D.T. (S ≥ 16 mm, R ≤ 14 mm).

3a) Salmonella/Citrobacter (4 hours)

	PYR
Salmonella spp.	0
Citrobacter spp.	+

© Copyright Rosco Diagnostica A/S

3b) Enterobacteriaceae (4 hours or overnight)

	PYR
Citrobacter, Klebsiella, Enterobacter, *Serratia* spp., and most *Yersinia* spp.	+
Edwarsiella. *E. coli*, Shigella, Salmonella, *Hafnia* spp., and all the Proteae	0

4) Arcanobacterium

	PYR	α–MAN	VP (24h)	TRIB	XYL
A. pyogenes	82	0	+	0	+
A. haemolyticum	0	+	0	70	0

5) Vancomycin resistant lactic cocci/coccobacilli from humans

	PYR	BE	ADH	Van5
Enterococcus	+	+	$+^0$	S^R
Pediococcus	0	+	+	R
Leuconostoc	0	$+^0$	0	R
Lactobacillus confusus	0	0	+	R

PYR = Pyrrolidonyl Aminopeptidase D.T., α–MAN = Alpha-Mannosidase D.T., VP(24h) = Voges-Proskauer D.T. (incubation 24 h), TRIB = Tributyrin D.T. and XYL = Xylose D.T., BE = Bile Esculin D.T., ADH = Arginine Dihydrolase D.T., Van5 = Vancomycin 5 µg Neo-S (S ≥15 mm, R ≤13 mm).

6) Differentiation of H_2S positive (TTR +) members of Enterobacteriaceae

	PYR	LDC	ARA	URE	ONPG
Citrobacter spp.	+	0	+	V	+
Edwardsiella tarda	0	+	0	0	0
Leminorella spp.	·	0	+	0	0
Proteus spp.	0	0	0	+	0
Salmonella subsp. I	0	+	+	0	0
Trabulsiella guamensis	0	+	+	0	+

PYR = Pyrrolidonyl Aminopeptidase D.T., LDC = Lysine Decarboxylase D.T., ARA = Arabinose D.T., URE = Urease D.T., TTR = Tetrathionate Reductase D.T.

7) Most common resistant non-fermenters

	TRIB	PYR	TRYP	ACM	TTR	ADH	COL	PSAER	Remarks
Ps. aeruginosa	$+^0$	+	+	+	+	+	S	R	
Ps. fluorescens	0^+	62	+	0^+	0^+	+	S	S	
Ps. putida	0^+	0	+	0	0	+	S	S	
Achr. xylosoxidans	0	+	0	$+^0$	+	0	69	S	
Alc. faecalis	0	0	0	+	+	0	S	S	DEFRX S
Burkh. cepacia complex	+	0	0	$+^0$	0	0	R	S	
Acin. baumanii (OXI 0)	+	0	0	0	0	0	S^R	S	NO_3 0, ß-XYL $+^0$
St. maltophilia (OXI 0)	+	0	+	0	$+^0$	0	V	S	IMIP R, α-MAN +, LDC +

TRIB = Tributyrin D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., ACM = Acetamide Hydrolysis D.T., TTR = Tetrathionate Reductase D.T., ADH = Arginine Dihydrolase D.T., COL = Colistin 10 µg D.T. (S ≥ 12 mm), PSAER = Ps. aerigunosa Screen D.T. (R ≤ 14 mm), NO_3 = Nitrate Reduction D.T., ß-XYL = Beta-Xylosidase D.T., α-Man = Alpha-Mannosidase D.T., IMIP = Imipenem Neo-S, LDC = Lysine Decarboxylase D.T., DEFRX = Deferoxamine D.T. (S ≥ 16 mm, R ≤ 14 mm).

© Copyright Rosco Diagnostica A/S

8) Differentiation between *Burkholderia*, *Ralstonia* and *Pandoraea* spp. (Colistin R)

	OXI	PYR	LDC	ONPG	URE	Alk P	Remarks
Burkholderia cepacia complex	+	0	$+^{0}$	$+^{0}$	V	$+^{0}$	
Ralstonia spp.	+	+	0	0	+	0	
Pandoraea spp.	67	·	0	0	+	+	Merop R, Genta R, Tobra R, LAP +, CAT +, MOT +
Burkholderia gladioli	0	0	0	+	$+^{0}$	67	

OXI = Oxidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., LDC = Lysine Decarboxylase D.T., URE = Urease D.T., Alk P = Alkaline Phosphatase D.T., Merop = Meropenem Neo-S, Genta = Gentamicin Neo-S, Tobra = Tobramycin Neo-S, LAP = Leucine Aminopeptidase D.T., CAT = catalase, MOT = motility.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Pyrrolidonyl Aminopeptidase (L-Pyrrolidonyl-ß-Naphthylamide)	*Enterobacter cloacae* ATCC 13047	*E. coli* ATCC 25922

References (PYR)

1) Wellstood S.A.: Rapid, Cost-Effective Identification of Group A Streptococci and Enterococci by Pyrrolidonyl-beta-Naphthylamide Hydrolysis. J. Clin. Microbiol. **125**, 1805-1806, 1987.
2) Mulczyk M., Szewczuk A.: Pyrrolidonyl peptidase in bacteria: a new colorimetric test for differentiation of Enterobacteriaceae. J. Gen. Microbiol. **61**, 9-13, 1970.
3) Casals J.B., Pringler N.: The value of 3 tests in the identification of staphylococci: pyrrolidonyl aminopeptidase (PYR) and susceptibility towards polymyxins and furazolidone. Staphylococci Symposium. Society Appl. Bacter. Edinburgh, July 1989.
4) Mackey T. et al: Identification of vancomycin - resistant lactic acid bacteria isolated from humans. J. Clin. Microbiol. **31**, 2499-2501, 1993.
5) Chagla A.H. et al: Evaluation of the l-Pyrrolidonal-β-NA hydrolysis Test for the differentiation of members of the families Enterobacteriaceae and Vibrionaceae. J. Clin. Microbiol. **31**, 1946-8, 1993.
6) Devriese L.A. et al: Identification of Enterococcus species isolated from foods of animal origin. Intl. J. Food Microbiol. **26**, 187-197, 1995.
7) Mahoudeau I. et al: Frequency of isolation of Staph. intermedius from humans. J.Clin. Microbiol. **35**, 2153-4, 1997.
8) Kahlmeter G. et al: S. lugdunensis- orsakar inte bara endokardit, 1998.

© Copyright Rosco Diagnostica A/S

3.3.5 TRYPSIN (BAA) (TRYP)

REF No. 47211

The test is based on enzymatic release of beta-naphthylamine from the benzoyl-arginin-beta-naphthylamide substrate. Beta-naphthylamine is detected by its reaction with Aminopeptidase reagent giving a red colour. The test is equivalent to the benzile arginine arilmidase (BAA) test.

Procedure
Prepare a dense bacterial suspension with a turbidity of at least McFarland No. 4 of the strain to be tested in 0.25 ml saline in a tube. Add one Trypsin tablet and close the tube. Incubate at 35-37 °C for **4 hours**.
After incubation add 3 drops of Aminopeptidase Reagent (92231) and read the colour reaction within 5 minutes.
See also Aminopeptidase, general description, page 12.

Reading of the tests (4h)
Positive reaction: **Red/orange**
Negative reaction: Yellow

Results

1) Porphyromonas

	TRYP	α-FUC	IND
P. asaccharolytica	0^{+}	$+^{0}$	$+^{0}$
P. gingivalis	+	0	+
P. endodentalis	0	0	+
P. catoniae	+	+	0

Downes et al (2) use the following Rosco D.T. in the identification of anaerobic gram-negative bacilli: α-FUC, NAG, β-XYL, α-GLU, TRYP, ESC, ONPG and URE.

2) Capnocytophaga spp. (Vanco 5 R, Kana 500 S, Colistin R, Trypsin $+^{0}$)

	OXI CAT	TRYP	β-XYL	NAG	$N0_3$	Remarks
C. gingivalis	0	+	0	0	0	
C. sputigena	0	+	+	$+^{0}$	$+^{0}$	
C. haemolytica	0	0	.	.	+	
C. ochracea	0	+	0	+	0	
C. granulosa	0	0	.	.	0	
C. cynodegmi (DF-2-like)	+	+	.	+	+	ADH +, PYR +, SUC +
C. canimorsus (DF-2)	+	+	.	+	0	ADH +, SUC 0

© Copyright Rosco Diagnostica A/S

3) IDENTIFICATION OF NON-FERMENTERS, where TRYP (BAA) is a major test (5)

	PYR	TRYP	TRIB	αMAN	LDC	IMP	Remarks
A) OXI 0							
Stenotrophomonas maltophilia	0	+	$+^0$	+	+	R	TTR $+^0$

	ACM	ALkP	α-GLU	TTR	ADH	DEF(S)	COL(S)	Remarks
B) OXI +, PYR +, TRYP +								
Ps. aeruginosa	+	3	0	+	+	R	100	PSAER (R)
Ps. fluorescens	0^+	0	0	0^+	+	R	100	PSAER (S)
Sh. putrefaciens	0	100	30	+	0	R	100	
Sh. algae	0	+	·	+	0	R	R	
Elisab. meningoseptica	0	100	+	0	0	R	R	IND $+^0$
Sphing. paucimobilis	0	100	+	0	0	R	19	IND 0, ß-XYL +, URE 0, Pigm +
Sphing. multivorum	0	100	+	0	0	R	R	IND 0, URE +
O. anthropi	0	0	V	·	36	R	93	
Inquilinus limosus	·	100	V	·	0	·	R	IND 0, PRO +, NAG + ß-GLU +, NO_3 0, ONPG +, res, mucoid
C) OXI +, PYR +, TRYP 0, NO_3 +								
Ralstonia picketii	0	0	0	0	0^+	100	R	MAN 0, NO_3 +
Ralstonia mannitolilytica	0	0	0	0	0	R	R	NO_3 0, MAN +
Com. acidovorans	+	0	0	+	0	R	R	PRO 0
Com. testosteroni	0	0	0	+	0	R	100	PRO 0
Achr. denitrificans	$+^0$	0	0	$+^0$	0	R	100	PRO +
Achr. xylosoxidans	$+^0$	0	0	$+^0$	0	R	69	PRO 0^+, MOT +, XYL +
Achr. piechaudii	V	0	0	·	0	R	100	PRO 0, NO_3+, TRIB +
Burkh. gladioli	·	·	·	·	·	R	R	ONPG +, OXI 0, Pigm $+^0$
D) OXI +, PYR 0, TRYP +								
Ps. fluorescens (PYR 62)	0^+	0	0	0^+	+	R	100	
Sphing. paucimobilis	0	100	+	0	0	R	19	
Brev. diminuta	0	100	0	0	0	92	R	NO_3 0
Brev. vesicularis	0	100	+	0	0	100	R	NO_3 0
Ps. stutzeri	0 wk	0	$+^0$	V	0	R	100	
Ps. alcaligenes	0	0	0	0	+	59	100	NO_3 +, PRO 0
Ps. pseudoalcaligenes	0	0	0	0	+	·	100	PRO +
Ps. putida	0	0	0	0	+	R	100	NO_3 0
E) OXI +, PYR 0, TRYP 0								
Burkh. cepacia complex ***)**	$+^0$	87	30	0	0	13	R	LDC $+^0$, ONPG $+^0$
Alc. faecalis	+	3	0	$+^0$	0	100	100	
Bord. bronchiseptica	0	0	0	0	0	R	100	
Olig. ureolytica	0	0	0	V	0^+	·	100	γ–GLU +, URE$+^R$, NO_3 +
Olig. urethralis	0	0	0	0	0	100	100	γ–GLU +, URE 0, NO_3 0
Pandoraea spp.	0	+	0	0	V	·	R	Merop R, LDC 0, ONPG 0, LAP +, CAT +, MOT +

TRYP = Trypsin D.T., α–FUC = Alpha-Fucosidase D.T., β-XYL = Beta-Xylosidase D.T., NAG = Beta-N-Acetyl-glusosaminidase D.T., NO_3 = Nitrate Reduction D.T., IND = Indole D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., TRIB = Tributyrin D.T., αMAN = Alpha-Mannosidase D.T., LDC = Lysine Decarboxylase D.T., IMP = Imipenem Neo-S (R = no zone), ACM = Acetamide Hydrolysis D.T., Alk P = Alkaline Phosphatase D.T., α–GLU = Alpha-Glucosidase D.T., TTR = Tetrationate Reductase D.T., ADH = Arginine Dihydrolase D.T., DEF = Deferoxamine D.T. (S ≥ 16 mm, R ≤ 14 mm), COL = Colistin 10 µg D.T. (S ≥ 13 mm, R ≤ 10 mm), PSAER = Ps. aeruginosa Screen D.T.

© Copyright Rosco Diagnostica A/S

(S ≥ 16 mm, R ≤ 14 mm), MAN = Mannitol D.T., NO_3 = Nitrate Reduction D.T., PRO = Proline Aminopeptidase D.T., VP = Voges Proskauer D.T., TTR = Tetrathionate Reductase D.T. , OXI = Oxidase D.T., CAT = catalase, SUC = Sucrose D.T., MOT = motility, URE = Urease D.T., Merop = Meropenem Neo-S, ONPG = ONPG Beta-Galactosidase D.T., LAP = Leucine Aminopeptidase D.T.
res = multiresistant.

***) *Burkholderia cepacia* complex (PYR 0, TRYP 0) and similar organisms**

Most strains are GLU +, ADH 0, OXI +wk.

	NO_3	LDC	ODC	42 °C	ONPG	PIGM	SUC	Remarks
B. cepacia genom (I)	4	100	30	43	100	82	91	
B. multivorans (II)	94	53 (V)	0	100	98	2	0	
B. cenocepacia (III)	31	99	71	84	99	17	90	
B. stabilis (IV)	4	100	100	0	0	0	0	
B. vietnamensis (V)	47	100	0	100	100	0	94	
B. dolosa (VI)	.	0	0	+	+	.	0	
B. ambifaria (VII)	V	100	0	26	100	V	95	ß haem 84
B. antina (VIII)	V	V	0	V	V	0	V	cream colonies
B. pyrrocinia (IX)	$+^0$	+	0	·	0	·	$+^0$	XYL +, MAL +, LACT + PRO +, NAG $+^0$
B. ubonensis (X)	·	·	·	·	·	·	·	
Pandoraea spp.	11	0	0	89	0	0	0	Alk P +, LAP +, CAT +, MOT +, MAL 0, Merop R,
B. gladioli	33	0	0	4	100	77	0	OXI 0, COL R, PYR +
R. picketti	17	0	0	83	0	0	0	Alk P 0, PYR +, DEFRX S, MAL +
B. fungorum	+	0	0	0	0	0	0	Alk P +, LAP +, CIT +, TRIB $+^0$

Note: Most common (> 80 %) in cystic fibrosis patients are *B. multivorans* and *B. cenocepacia* (6).

NO_3 = Nitrate Reduction D.T., LDC = Lysine Decarboxylase D.T., ODC = Ornithine Decarboxylase D.T., 42 °C = growth at 42 °C, PIGM = Pigment production (brown or yellow), SUC = Sucrose D.T., MAL = Maltose D.T., OXI = Oxidase D.T., XYL = Xylose D.T., LACT = Lactose D.T., PRO = Proline Aminopeptidase D.T., NAG = Beta-N-Acetylglucosaminidase D.T., COL = Colistin 10 µg Neo-S (S ≥ 13 mm, R ≤ 10 mm) DEFRX = Deferoxamine D.T. (S ≥ 16 mm, R ≤ 14 mm). GLU = Glucose D.T., ADH = Arginine Dihydrolase D.T., Alk P = Alkaline Phosphatase D.T., LAP = Leucine Aminopeptidase D.T., CAT = catalase, MOT = motility, Merop = Meropenem Neo-S, CIT = Citrate D.P., TRIB = Tributyrin D.T.

4) Differentiation of *Ps. fluoresens*, *Ps. putida*, and *Ps. stuzeri* (Tryp +)

	PYR	α-GLU	TRIB
P. fluorescens	V	0	+
P. putida	0	0	0
P. stutzeri	0	$+^0$	+

PYR = Pyrrolidonyl Aminopeptidase D.T., α-GLU = Alpha-Glucosidase D.T., TRIB = Tributyrin

5) Differentiation of *Burkh. cepacia* complex from *B. gladioli*, *Ralstonia pickettii* and *R. manitolilytica*

	PYR	OXI	ONPG	DEF	COL	
Burkh. cepacia complex	0	+ wk	$+^0$	R	R	
B. gladioli	+	0	+	R	R	
R. pickettii	+	+	0	S	R	
R. manitolilytica	+	+	0	R	R	MAN +

PYR = Pyrrolidonyl Aminopeptidase D.T., OXI = Oxidase D.T., ONPG = ONPG D.T., DEF = Deferoxamine D.T., COL = Colistin D.T.

© Copyright Rosco Diagnostica A/S

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Trypsin (Na-Benzoyl-DL-Arginine-ß-Naphthylamide)	*S. maltophilia* ATCC 13637	*E. coli* ATCC 25922

References (TRYP)

1) Summanen P. et. al: Wadsworth Anaerobic Bacteriology Manual. 5th. Ed. Advanced Identification Methods (Level III) pages 49, 50, 65, 93, 158-159 (1993).
2) Downes J. et al: Evaluation of the Rapid ID 32 A system for identification of anaerobic Gram-negative bacilli, excluding the Bacteroides fragilis group. Clin. Microbiol. and Infect. **5**, 319-326, 1999.
3) Henry D.A.: Phenotypic methods for determining genomovar status of the Burkholderia cepacia complex. J. Clin. Microbiol. **39**, 1073-8, 2001.
4) Coenye T. et al: Taxonomy and identification of the Burkholderia cepacia complex. J. Clin. Microbiol. **39**, 3427-36, 2001.
5) Laffineur K. et al: Biochemical and susceptibility tests useful for identification of non-fermenting gram negative rods. J. Clin. Microbiol. **40**, 1085-7, 2002.
6) Reik R. et al: Distribution of Burkholderia cepacia complex species among isolates recovered from persons with or without cystic fibrosis. J. Clin. Microbiol. **43**, 2926-8, 2005.

© Copyright Rosco Diagnostica A/S

3.4 Anaerobes, Presumptive Identification with Oxgall (bile), Brilliant Green and Antibiotic Tablets

A simple screening method is described for separating the major groups of common anaerobic bacteria.

Procedure
Oxgall D.T. (bile) (44311), Brilliant Green D.T. (40511) and the antibiotic tablets: Vancomycin 5µg Neo-Sensitabs (45111), Kanamycin 500 µg Neo-Sensitabs (43111), Colistin 10 µg D.T. (41811), and Rifampicin 30 µg Neo-Sensitabs (26112) are placed on a plate of FAA + 5% blood or supplemented Brucella Blood Agar, which has been inoculated with an inoculum corrsponding to 0.5 McFarland. The plates are incubated anaerobically and the inhibition zones are read after **24-48 hours**.

Results

	CAT	Oxgall (bile)	Brilliant Green	Vanco 5 µg	Kana 500 µg	Colistin 10 µg	Rifa Neo-S	Fosfo Neo-S	MOT	Remarks
Bact.fragilis group	V	R	S	R	R	R	S	R	0	
Prev.melaninogen./oralis	0	S	S	R	R	S^R	S	R	0	
Porphyromonas spp.	0	S	S	S	R	R	S	R	0	
*Bact.ureolyticus**	0	S	S	R	S	S	V	.	0	
Fusob.mortiferum/varium	0	R	R	R	S	S	R	S	0	
Other Fusobacteria	V	V	R	R	S	S	V	S	0	
Bilophila wadsworthia	+	R	.	R	S	S	.	.	+	SIM
Gram positive cocci	.	.	.	S	V	R	S	R	.	
Gram negative cocci	.	S^R	.	R	S	S	S	.	.	
Clostridia spp.	.	V	.	S	V	R	S^R	R	.	
Prevotella massiliensis	0	.	.	R	S	.	.	.	.	OXI +
Sutterella wadsworthiensis	0	R	.	R	S	S	.	.	0	
Dialister pneumosintes	0	S	.	R	S	R	.	.	.	
Synergistes spp.	0	R^S	.	R	S	R	.	.	.	

R = resistant, **S** = sensitive, $\mathbf{S}^R$ = most strains sensitive, **V** = variable, CAT = Catalase, OXI = Oxidase, MOT = motility.

***** *Bact.ureolyticus* is nitrate and urease positive.

For Brilliant Green, Kanamycin 500 µg, and Colistin 10 µg: **Sensitive ≥10 mm**; Resistant <10 mm.
For Vancomycin 5 µg: **Sensitive ≥20 mm**; Resistant <18 mm.
For Rifampicin 30 µg Neo-Sensitabs: **Sensitive ≥16 mm**; Resistant <16 mm.
For Oxgall (bile): **Sensitive: any zone**; Resistant: no zone.

The Oxgall tablets, after incubation, are normally surrounded by a large zone of hemolysis. Organisms growing within this zone of hemolysis (resistant to oxgall) often produce a cloudy precipitate in the agar medium.

Screening of gram negative anaerobes:

	Vancomycin 5 µg	Kanamycin 500 µg	Colistin 10 µg	Fosfomycin	Remarks
Bact. fragilis group	R	R	R	R	OXI 0
Prevotella spp.	R	R	S^R	R	OXI 0
Porphyromonas spp.	S	R	R	R	OXI 0
Fusobacterium spp.	R	S	S	S	OXI 0
Prevotella massiliensis	R	S	.	.	OXI +

© Copyright Rosco Diagnostica A/S

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
Oxgall 1000 µg (Oxgall)	*S. aureus* ATCC 25923	*B. fragilis* ATCC 25285
Brilliant Green 100 µg	*B. fragilis* ATCC 25285	*F. necrophorum* ATCC 25556
Colistin 10 µg (Colistin sulphate)	*E. coli* ATCC 25922	*S. aureus* ATCC 25923

References

1) Draper D.L., Barry A.L.: Rapid identification of Bacteroides fragilis with bile and antibiotic disks. J. Clin. Microbiol. **5**, 439-443, 1977.
2) Leigh D.A., Simmons K.: Identification of non-sporing anaerobic bacteria. J. Clin. Pathol. **30**, 991-992, 1977.
3) Halebian S. et al: Rapid method that aids in distinguishing Gram-positive from Gram-negative anaerobic bacteria. J. Clin. Microbiol. **13**, 444-448, 1981.
4) Murray P.R., Citron D.M.: General Processing of Specimens for Anaerobic Bacteria, pp. 488-504 (499-500) in "Manual of Clinical Microbiology" 5th ed., Balows et al (eds.), ASM, 1991.
5) Bernard D. et al: Bilophila wadsworthia bacteremia in a patient with gangrenous appendicitis. CID, **18**, 1023-4, 1994.
6) Anaerobic Gram-negative bacteria, p. 888-896 in Manual of Clinical Microbiology 8th ed. Yolken R.H. et al (eds), ASM 2003.

© Copyright Rosco Diagnostica A/S

3.5 ARGININE DIHYDROLASE (ADH)

REF No. 56211

L-arginine is broken down in a two step process: first from l-arginine to l-citrulline (ADH) followed by a citrulline splitting system. The over-all reaction results in the formation of l-ornithine, CO_2 and NH_3 from the substrate l-arginine, resulting in an alcalinization of the medium and a change of colour of the indicator from yellow to red.

Procedure
Prepare a dense "milky" suspension (at least McFarland No. 4) from the strain to be tested in 0.25 ml saline in a tube. Add one ADH Diagnostic Tablet and **3 drops of sterile paraffin oil**. Close the tube and incubate at 35-37 °C **for 4 hours** or **up to 18-24 hours**.

Reading of the test
Positive reaction: **Red**
Negative reaction: Yellow

After **overnight** incubation, positive reaction: **strong red**; negative reaction: yellow or orange. In most cases overnight incubation is necessary.

Results

1) Enterobacter

Positive: *Enterobacter cloacae*
Usually negative: Other *Enterobacter* spp.

	ADH	MR	Remarks
E. cloacae	97	5	
E. aerogenes	0	5	
E. intermedium	0	100	
E. sakazakii	99	5	α-GLU +
E. agglomerans	0	50	ODC 0

ADH = Arginine Dihydrolase D.T., MR = Methyl Red, α-GLU = Alpha-Glucosidase D.T., ODC = Ornithine Decarboxylase D.T.

2) Streptococci/Enterococci

Positive: *E. faecalis*, *E. faecium*, *E. durans*, *E. gallinarum*, *E. casseliflavus*.
Negative: Group D streptococci (*Strept. bovis*, *Strept. equinus*) *E. avium*, *E. raffinosus*.

3) Non-fermenters

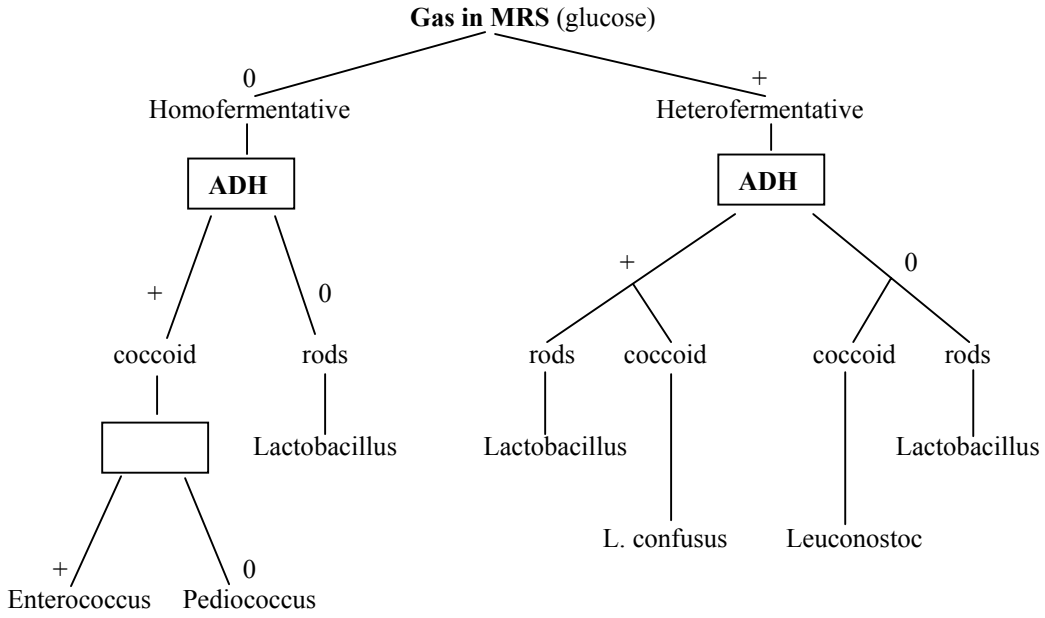
Positive: *Ps. aeruginosa*, *Ps. fluorescens*, *Ps. putida*, *Ps. pseudoalcaligenes*, *Ps. alcaligenes*, *Ps. stutzeri*, *Cryseom. luteola* (Ve-1).
Negative: *St. maltophilia*, *Sphing. paucimobilis*, *Shew. putrefaciens*, *Flavobacterium* spp., *Brev. vesicularis*, *Com. acidovorans*, *Com. testosteroni*, *Pasteurella multocida*, *Ralst. pickettii*, *Alcaligenes* spp., *Brev. diminuta*, *Burkh. cepacia*, *Oligella* spp.

4) Staphylococci

Usually positive: *S. aureus*, *S. haemolyticus*, *S. schleiferi*, *S. simulans*, S. warneri, *S. capitis*.
Usually negative: *S. hominis*, *S. lugdunensis*, *S. saprophyticus*, *S. xylosus*, *S. cohnii*, *S. sciuri*, *S. lentus*

© Copyright Rosco Diagnostica A/S

5) Identification of lactic bacteria (Vancomycin R)



6) Differentiation of NVS (*Abiotrophia* and *Granulicatella* spp (4))

	ADH	PGUA	NAG	α-GAL
Abiotrophia defectiva	0	0	0	+
Gran. adjacens	0	$+^0$	0	0
Gran. elegans	+	0	0	0
Gran. balaenopterae	+	0	+	.

NVS = nutritionally variant streptococci, ADH = Arginine Dihydrolase D.T., PGUA = Beta-Glucuronidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., MR = methyl red, MRS = Man, Sharp, Rogosa broth, NAG = N-Acetyl-glucosaminidase D.T., α-GAL = Alpha-Galactosidase D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Arginine Dihydrolase (L-Arginine HCl)	*P. aeruginosa* ATCC 27853	*K. pneumoniae* ATCC 13883

References

1) Mackey T. et al: Identification of Vancomycin-resistant lactic bacteria isolated from humans. J. Clin. Microbiol. **31**, 2499-2501, 1993.
2) Mohr O'Hara et al: Isolation of Enterobacter intermedium from the gallbladder of a patient with cholecystitis. J. Clin. Microbiol. **36**, 3055-6, 1998.
3) Sato S. et al: Abiotropia elegans comprise 8% of the nutritionally variant streptococci isolated from the human mouth. J. Clin. Microbiol. **37**, 2553-6, 1999.
4) Christensen J.J., Facklam R.R.: Granulicatella and Abiotrophia species from human clinical specimens. J. Clin. Microbiol. **39**, 3520-3, 2001.

© Copyright Rosco Diagnostica A/S

3.6 BACITRACIN LOW (BaL)

REF No. 40211

Contain a lower amount of bacitracin (0.4 units) than Bacitracin Neo-Sensitabs, and are specially intended for differentiation of the Lancefield **group A beta haemolytic streptococci** from other **beta-haemolytic streptococci**.

The test is performed on TSA Blood Agar inoculated with the strain to be tested (growth just confluent).

Bacitracin Low Diagnostic Tablets will with group A beta-haemolytic streptococci produce inhibition zones: **>15mm**, while most beta-haemolytic streptococci from other groups will show smaller or no inhibition zones. Some false sensitive results are seen mainly with streptococci group C and G.

Results

1) Streptococci

Group A streptococci: >15 mm
Other streptococci: <14 mm

Bacitracin resistant clones of *S. pyogenes* (group A) were isolated from Belgian and Spanish patients (3,4).
Confirm *S. pyogenes* using the PYR test.
Most bacitracin resistant *S. pyogenes* (A) are resistant to erythromycin and clindamycin.

2) *Gardnerella vaginalis*

The test is performed on Mueller-Hinton II agar + 5% blood with an inoculum equivalent to McFarland 0.5

Gardnerella vaginalis: ≥ 10 mm, PRO +
Bifidobacteria: < 10 mm
Lactobacilli: < 10 mm
Streptococci: < 10 mm

3) Throat cultures

	BaL	PYR	MUPIR	OPT	
Arcanobact. haemolyticum	R	0	R	R	(≤ 16 mm)
Strept. pyogenes A	S^R	+	S	R	
Strept. group C/G	R^S	0	S	R	
Pneumococci	R	0	S	S	(≥ 18 mm)

BaL = Bacitracin low D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., MUPI = Mupirocin Neo-S (S ≥ 16 mm, R < 16 mm), OPT = Optochin D.T.

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
Bacitracin low 0.4 U	*S. pyogenes* ATCC 12344	*S. bovis* ATCC 15351

References

1) Stoner R.A.: Bacitracin and coagglutination for grouping of beta-haemolytic streptococci. J. Clin. Microbiol. **7**, 463-466, 1978.
2) Bellon J., Weise B., Verschraegen G., de Meyere M.: Selective Streptococcal Agar Versus Blood Agar for Detection of Group A Beta- Hemolytic Streptococci in Patients with Acute Pharyngitis. J. Clin. Microbiol. **29**, 2084-2085, 1991.
3) Malhotra-Kumar S. et al: Bacitracin-resistant clone of Streptococcus pyogenes isolated from pharyngitis patients in Belgium. J. Clin. Microbiol. **41**, 5282-4, 2003.
4) Montes M. et al: Characterization and evolution of a macrolide and bacitracin-resistant S. pyogenes clone in Spain: 1999-2005. 46th ICAAC, abstract C2-0201, 2006.

© Copyright Rosco Diagnostica A/S

3.7 BACITRACIN 40 UNITS (BACIT) Neo-Sensitabs

REF No. 70812

Chocolate blood-agar with a Bacitracin 40 units Neo-Sensitabs is useful for the isolation of *Haemophilus* spp. in sputum samples. The test is based on the resistance of *Haemophilus* spp. to high concentrations of bacitracin. Gram positive cocci will show large zones of inhibition around the Bacitracin 40 units tablet, while Haemophilus strains grow near the edge of the tablet (1,2).

Results

BACITRACIN 40 U	**Screening of *Haemophilus* spp. in throat/sputum cultures**
Haemophilus spp.	Growth **very near** the tablet edge
Streptococci/Staphylococci	Growth **far** from the tablet

References

1) Möller L.V.M. et al: N-acetyl-d-glucosamine medium improves recovery of *H. influenzae* from sputa of patients with cystic fibrosis. J. Clin. Microbiol. **31**, 1952-4, 1993.
2) Nye K.S. et al: Incorporated chocolate blood agar and chocolate blood agar plus a bacitracin disk in the isolation of *H. influenzae* from sputum. J. Med. Microbiol. **50**, 472-5, 2001.

© Copyright Rosco Diagnostica A/S

3.8 BETA LACTAMASE (Acido)

REF No. 45521

The beta lactamase test (acidometric) is suitable for detecting the production of beta lactamase by the following strains: **Haemophilus**, ***Neisseria gonorrhoeae***, and **staphylococci**.
The test is based on the opening of the beta lactam ring of the substrate (penicillin G) by beta lactamase, resulting in an acidic compound which changes the colour of the indicator (bromcresol purple) from violet to yellow.

Procedure
Prepare a heavy (at least McFarland No. 4) bacterial suspension in 0.25 ml water or saline in a small tube by picking colonies of the test organism from an overnight plate. A Beta Lactamase Diagnostic Tablet is added. Incubate at 35-37 °C.

Reading of the tests
Positive reaction: The supernatant turns **yellow** (or brownish) within **15-20 min**.*
Negative reaction: Violet

* The reaction time may vary depending upon species, age of culture and the individual strain. A test should not be called negative unless no colour change has taken place in **4 hours**.

Beta Lactamase Induction
It should be noted that some **staphylococci** will not show beta lactamase production, unless the enzyme has been induced by exposure to a beta lactam antimicrobial. In such cases, use growth adjacent to beta lactam antimicrobial tablets (oxacillin, methicillin) or from agar containing beta lactams.

The use of the Beta Lactamase test with strains of Enterobacteriaceae is debatable, because there is lack of correlation between enzyme detection and resistance to beta lactam antibiotics, such as ampicillin, carbenicillin or cephalosporins.

Store at 2-8 °C. Before opening the vial, keep it at room temperature for 1 hour; after opening store at room temperature for up to 2 months.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Beta-Lactamase (Acido) (Penicillinprocaine 4 mg, Penicillin G sodium)	*S. aureus* ATCC 29231	*S. aureus* ATCC 25923

References
1) Shannon K., Phillips I.: Beta-lactamase by 3 simple methods: intralactam, nitrocefin and acidometric, J. Antimicrob. Chemother. **6**, 617-621, 1980.
2) Wegener H.C. et al: Antimicrobial susceptibility of Staph. hyicus isolated from exudative epidermitis in pigs. J. Clin. Microbiol. **32**, 793-5, 1994.

© Copyright Rosco Diagnostica A/S

3.9 Beta-lactamases (ESBL, AmpC, MBL) detection using Neo-Sensitabs and Diatabs

The detection of different resistance mechanisms in bacteria has in the last years been highlighted by many publications and national recommendations are available in many countries.
Antimicrobial resistance mechanisms including the beta-lactamases are continuously developing and new methods for detection are coming up. Rosco has a broad range of products that in combination may detect different beta-lactamases by phenotypic profiles.

Diatabs	**Code**	**REF No.**
Cloxacillin 500 µg Diatabs	CL500	10031
Boronic Acid 250 µg	BORON	10041
Ceftazidime+DPA 30+250 µg *		
Dipicolinic Acid 250 µg *	D.P.A	

* Pipeline products. For investigational and research use only.

Neo-Sensitabs - CLSI potencies New cartridges (cartridges with spring)	**Code**	**REF No.**
Aztreonam 30 µg	AZT30	63612
Cefepime 30 µg	FEP30	63712
Cefotaxime 30 µg	CTX30	63912
Cefoxitin 30 µg	CFO30	62912
Cefpodoxime 10 µg	CPD10	63212
Ceftazidime 30 µg	CAZ30	64012
Ceftriaxone 30 µg	CTR30	64212
Amoxycillin+Clavulanate 20+10 µg	AMC30	60112
Cefepime+Clavulanate 30+10 µg	FEP+C	64812
Cefotaxime+Clavulanate 30+10 µg	CTX+C	64712
Cefpodoxime+Clavulanate 10+1 µg	CPD+C	80912
Ceftazidime+Clavulanate 30+10 µg	CAZ+C	64612
Imipenem 10 µg	IMI10	61212
Imipenem+EDTA 15+750 µg	IM+ED	80512N
Ticarcillin+Clavulanate 75+10 µg	TIM85	64412
Meropenem 10 µg	MRP10	64312

Neo-Sensitabs Cartridges without spring	**Code**	**REF No.**
Aztreonam 30 µg	AZTRM	70712
Cefepime 30 µg	CFEPM	71212
Cefoxitin 60 µg	CFOXT	71712
Ceftazidime 30 µg	CEZDI	72212
Ceftriaxone 30 µg	CETRX	72612
Amoxycillin+Clavulanate 30+10 µg	AM+CL	70212
Cefepime+Clavulanate 30+10 µg	CP+CL	79512
Ceftazidime+Clavulanate 30+10 µg	CZ+CL	72312
Imipenem 15 µg	IMIPM	74612
Imipenem+EDTA 15+750 µg	IM+ED	80512
Ticarcillin+Clavulanate 75+15 µg	TI+CL	78812
Meropenem 10 µg	MEROP	75312

© Copyright Rosco Diagnostica A/S

Detection of ESBLs using Neo-Sensitabs

1) Enterobacteriaceae (Fig 1)
Strains showing cefotaxime and/or ceftazidime MICs ≥ 2 µg/ml, showing reduced susceptibility to amoxycillin + clavulanate should be tested further for the presence of ESBLs.
Mueller-Hinton agar plates are inoculated with the strain to be tested, and Neo-Sensitabs applied onto the agar; Cefotaxime, Ceftazidime and Cefepime Neo-Sensitabs at a distance of approx. 20 mm (edge to edge) from Amoxycillin + Clavulanate Neo-Sensitabs or using their combinations: Cefotaxime + Clavulanate, Cefepime + Clavulanate and Ceftazidime + Clavulanate Neo-Sensitabs.
A keyhole or ghost (synergy) zone between Amox + Clav and any of Cefotaxime, Ceftazidime or Cefepime Neo-Sensitabs indicates the presence of an ESBL.
When using the combination disks, a ≥ 5 mm larger zone for any combination compared to the corresponding single antimicrobial indicates the presence of an ESBL. Always use products from the same range with and without clavulanic acid, e.g. cephalosporins in new cartridges must only be compared to same antimicrobial with clavulanic acid in new cartridges.
Cefpodoxime and Cefpodoxime + Clavulanate may be used for screening purposes.
Klebsiella oxytoca hyperproducing K-1 beta-lactamase may show a false positive result (potentiation of cefotaxime and/or cefepime). Only when the strain is resistant to ceftazidime and shows synergism between ceftazidime and clavulanate should it be reported as ESBL positive.

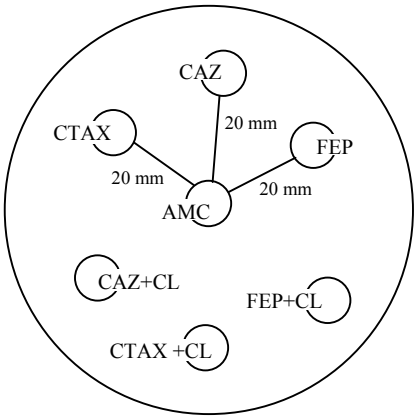


Fig 1. ESBL - Enterobacteriaceae

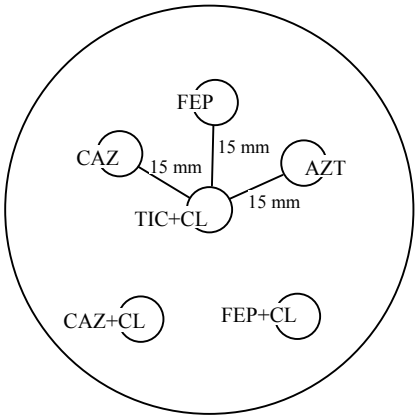


Fig 2. ESBL - Non-fermenters

CTAX Cefotaxime, CTAX+CL Cefotaxime+Clavulanic acid, CAZ Ceftazidime, CAZ+CL Ceftazidime+Clavulanic acid, FEP Cefepime, FEP+CL Cefepime+Clavulanic acid, AMC Amoxycillin+Clavulanic acid, AZT Aztreonam, TIC+CL Ticarcillin+ Clavulanic acid.

2) Non-fermenters (Fig 2)
Particularly *P. aeruginosa* and *A. baumannii* may possess several types of beta-lactamases. Non-fermenters showing reduced susceptibility to ceftazidime and/or cefepime and/or aztreonam should be tested for the presence of ESBLs. Apply Ceftazidime, Cefepime and Aztreonam Neo-Sensitabs. At a distance of approx. 15 mm (edge to edge) from them apply Ticarcillin + Clavulanate Neo-Sensitabs. Separately from them apply Ceftazidime + Clavulanate and Cefepime + Clavulanate Neo-Sensitabs.
A keyhole zone or ghost zone (synergism) between Ticarcillin + Clavulanate and any of Ceftazidime, Cefepime or Aztreonam Neo-Sensitabs indicates the presence of an ESBL
With the combination disks a ≥ 5 mm larger zone for Ceftazidime + Clavulanate or Cefepime + Clavulanate compared to the single antimicrobials indicates the presence of an ESBL. Always use products from the same range with and without clavulanic acid, e.g. cephalosporins in new cartridges must only be compared to same antimicrobial with clavulanic acid in new cartridges.

© Copyright Rosco Diagnostica A/S

Detection of AmpC Beta-lactamases using Neo-Sensitabs and Diatabs

Enterobacteriaceae (Fig 3)
Strains suspicious of possessing plasmid-mediated AmpC beta-lactamases are cefoxitin resistant and have reduced susceptibility to ceftazidime, while currently they are susceptible to cefepime and the carbapenems.
Apply Ceftazidime and Cefoxitin Neo-Sensitabs. At a distance of 10 mm (edge to edge) from each apply Cloxacillin 500 ug Diatabs. Apply Ceftazidime + Clavulanate and Cefotaxime + Clavulanate Neo-Sensitabs. At a distance of 10 mm (edge to edge) apply Boronic Acid Diatabs.
A keyhole or ghost zone (synergism) between Cloxacillin 500 µg and any of Ceftazidime or Cefoxitin indicates the presence of an AmpC beta-lactamase.
A keyhole or ghost zone (synergism)between Boronic Acid and any of Ceftaxime+Clavulanate or Cefepime + Clavulanate, indicates the presence of an AmpC beta-lactamase.
Inducible AmpC beta-lactamases will show antagonism (distorted zone) between Cefoxitin and Ceftazidime Neo-Sensitabs. Strains producing plasmid-mediated inducible AmpC enzymes will also show antagonism between cefoxitin and ceftazidime.
In order to differentiate plasmidic AmpC and chromosomal AmpC, look at the zones around Cefotaxime, Ceftazidime and Aztreonam Neo-Sensitabs. The presence of scattered colonies (resistant mutants) at the edge of the zones indicates plasmid-mediated AmpC, while the presence of well-defined zone-edges indicates chromosomal beta-lactamase.

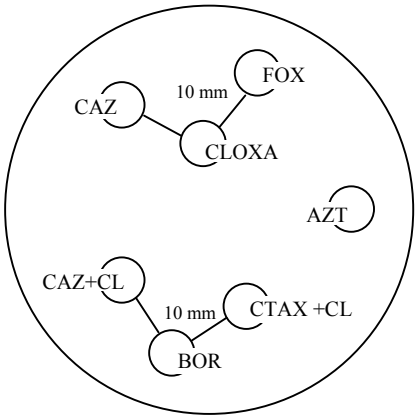


Fig 3. AmpC

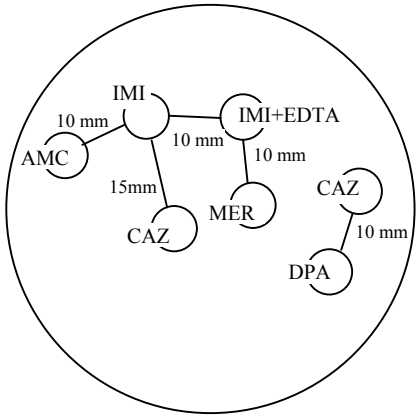


Fig 4. Metallo-Beta-Lactamases + Carbapenemases

FOX Cefoxitin, CLOXA Cloxacillin, CAZ Ceftazidime, AZT Aztreonam; CAZ+CL Ceftazidime+Clavulanic acid, CTAX+CL Cefotaxime+Clavulanic acid BOR Boronic acid, AMC Amoxycillin+Clavulanic acid, IMI Imipenem , IMI+EDTA Imipenem+EDTA, MER Meropenem, DPA Dipicolinic acid.

Detection of Carbapenemases using Neo-Sensitabs and Diatabs

Test only strains resistant to ceftazidime (inhibition zone < 16 mm), because strains susceptible to ceftazidime do not produce carbapenemases. Most of the strains show also some kind of reduced susceptibility to carbapenems (imipenem/meropenem MICs ≥ 2 µg/ml).

1) Metallo-beta-lactamases (Fig 4)
Apply Imipenem and Imipenem + EDTA Neo-Sensitabs at a distance of 10 mm from each other. Apply Meropenem at a 10 mm distance from Imipenem + EDTA.
Apply Ceftazidime Neo-Sensitabs and Dipicolinic acid Diatabs at a distance of 10 mm from each other.
A keyhole or ghost zone (synergism) between Ceftazidime and Dipicolinic acid indicates the presence of a metallo-beta-lactamase. This technique is the most recommended.
A difference of ≥ 5 mm larger zone with Imipenem + EDTA compared to Imipenem alone indicates the presence of a metallo-beta-lactamase. Some false positives may occur due to the activity of EDTA alone against some strains. Therefore a keyhole or ghost zone (synergism) between Imipenem and Imipenem + EDTA and/or between Imipenem + EDTA and meropenem is a good confirmatory test.

© Copyright Rosco Diagnostica A/S

2) Other Carbapenemases (Fig 4)
Strains showing a negative metallo-beta-lactamase test may produce other carbapenemases. They are Class A enzymes such as IMI, KPC-1 to KPC-4, GES-2 to GES-5, isolated from Enterobacteriaceae, particularly *Klebsiella pneumoniae*. Besides Class D enzymes, such as oxacillinases (OXA-23, OXA-24, OXA-51-OXA-58) isolated mainly from *A. baumannii*.
An assay for carbapenemase production is described in User's Guide Neo-Sensitabs (under detection of class A and D carbapenemases). Briefly: A MH plate is inoculated with *E. coli* ATCC 25922 and Imipenem and Meropenem Neo-Sensitabs applied onto the plate. A suspension of the organism to be tested is applied with a loop making a streak of 15-20 mm on each side of Imipenem/Meropenem Neo-Sensitabs. After incubation, a distortion of the zone of inhibition around the streaks indicates the presence of a carbapenemase.
Strains producing Class A enzymes are isolated more frequently. They show currently imipenem MICs ≥ 4 µg/ml and resistance to ceftazidime and aztreonam. In most cases, they will show synergy between imipenem and clavulanate (amox+clav).
Some Class A enzymes (NmcA, Sme-1 to Sme-3, IMI-1, IMI-2) are inducible and these strains will show antagonism between Imipenem and Ceftazidime
Class D enzymes (oxacillinases) are responsible for many *A. baumannii* outbreaks. They show currently imipenem/meropenem MICs ≥ 2 µg/ml and are ceftazidime resistant. Synergism between imipenem and clavulanate is weak or non-existent.

References
1) Vercauteren E. et al: Comparison of screening methods for detection of ESBLs and their prevalence among blood isolates of E. coli and Klebsiella spp. in a Belgian Teaching Hospital. J. Clin. Microbiol. **35**, 2191-2197, 1997.
2) de Gheldre Y. et al: National epidemiologic survey of Enterobacter aerogenes Belgian hospitals from 1996 to 1998. J. Clin. Microbiol. **39**, 889-896, 2001.
3) Tzelepi E. et al: Detection of ESBL in clinical isolates of Enterobacter cloacae and E. aerogenes. J. Clin. Microbiol. **38**, 542-546, 2000.
4) Mirelis B. et al: A simple phenotypic method for the differentiation between acquired and chromosomal AmpC beta-lactamases in E. coli. Enferm. Infecc. Microbiol. Clin. **24**, 370-372, 2006.
5) Wonkeun Song et al: Use of Boronic acid methods to detect the combined expression of plasmid mediated AmpC beta-lactamases and ESBLs in clinical isolates of Klebsiella spp., Salmonella and P. mirabilis. Diagn. Microbiol. Infect. Dis..**57**, 315-318, 2007.
6) Prof P. Nordmann: Evaluation de tests phenotypiques de detection de cephalosporinases integrant l'utilisation des disques de cloxacilline et d'acide boronique. Oct. 2006. Internal study.
7) Ruppe E. et al: First detection of the Ambler Class C1 AmpC beta-lactamase in Citrobacter freundii by a new simple double-disk synergy test. J. Clin. Microbiol. **44,** 4204-4207, 2006.
8) Cornaglia G. et al: Metallo-beta-lactamases as emerging resistance determinants in gram-negative pathogens: open issues. Int. J. Antimicr. Ag. **29**, 380-388, 2007.
9) Franklin C. et al: Phenotypic detection of carbapenem-susceptible metallo-beta-lactamase-producing gram-negative-bacilli. J. Clin. Microbiol. **44**, 3139-3144, 2006.
10) Moland E.S. et al: Prevalence of newer beta-lactamases in gram-negative clinical isolates collected in the U.S. from 2001 to 2002. J. Clin. Microbiol. **44**, 3318-3324, 2006.
11) Deshpande L.M. et al: Emergence of serine carbapenases (KPC and SME) among clinical strains of Enterobacteriaceae in the U.S. Medical Centers: Report from the MYSTIC Program (1999-2005). Diagn. Microbiol. Infect. Dis. **56**, 367-372, 2006.

© Copyright Rosco Diagnostica A/S

3.10 ESCULIN HYDROLYSIS (ESC) BILE ESCULIN (BE)

REF No. 56611
REF No. 40411

Both tests are based on the demonstration of esculetin released by hydrolysis of esculin. Esculetin reacts with iron to form a brown/black phenolic iron complex. The Bile Esculin Test is mainly used in **differentiating Group D streptococci** and **enterococci** (positive) **from other streptococci** (negative). Esculin Hydrolysis is useful in the differentiation of Streptococci, Enterobacteriaceae, non-fermenters, etc.

Procedure 1
Make a dense suspension of the strain to be tested in 0.25 ml physiological saline with a turbidity of at least McFarland No. 4 in a small tube. Add one Diagnostic Tablet and close the tube. Incubate a 35-37 °C for **4 hours** (or up to **24 hours**).

Reading of the tests
Positive reaction: **Black/grey**
Negative reaction: Colourless/light grey

Procedure 2
The Diagnostic Tablets are placed onto a blood agar plate inoculated with the strain to be tested. The plate is incubated at 35-37 °C **overnight**.

Reading of the tests
Positive reaction: The tablet and the colonies around it turn **black/grey** and there is no zone of inhibition (Bile Esculin).
Negative reaction: The tablet remains white and the colour of the colonies has not changed. A zone of inhibition may appear around the Bile Esculin tablet.

Results

1) *Yersinia enterocolitica* pathogenic serotype

	ESC	SAL	PZA
Yersinia enterocolitica (pathogenic serotype)	0	0	0
Yersinia enterocolitica (non pathogenic)	+	+	+
Yersinia spp.	V	V	+

ESC = Esculin Hydrolysis D.T., SAL = Salicin D.T. and PZA = Pyrazinamidase D.T. All tests performed at **25 °C**.

2) Identification of vancomycin resistant cocci/coccobacilli from humans

	BE	PYR	ADH	Van5
Enterococcus	+	+	$+^0$	S/R
Pediococcus	+	0	$+^0$	R
Leuconostoc	$+^0$	0	0	R
Lactobac. confusus	0	0	+	R

BE = Bile Esculin D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., ADH = Arginine Dihydrolase D.T. and Van5 = Vancomycin 5 µg Neo-S (S≥15 mm, R≤12 mm).

© Copyright Rosco Diagnostica A/S

3) Differentiation of *S. bovis* I/II, *S. mutans* and *E. faecalis*

	BE	PYR	SORB	MAN	α-GAL
S. gallolyticus (S. bovis I)	+	0	0	+	+
S. bovis II (*S. bovis)*	+	0	0	0	.
S. mutans	V	0	+	+	.
E. faecalis	+	+	+	$+^0$	0

BE = Bile Esculin D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., SORB = Sorbitol D.T., MAN = Mannitol D.T. α-GAL = Alpha-Galactosidase D.T.

4) Identification of Actinomyces and related species from human sources

Most strains are: Vanco 5 S, Kana 500 S^R, Col R, Metro R^S, Cipro R.

	PYR	PIGM	CAT	NO_3	CAMP	URE	ESC	αFUC	PGUA	αGLU	NAG	ONPG	ARA	Remarks
A. europaeus		0	0	+	0	0	+	0		+	0	+	0	SUC 0, RAF 0
A. dentalis		0	0	0	.	0	+	0		+	0	+	0	
A. funkei		0	0	+	+	0	0	+		+	+	+	+	
A. georgiae		0	0	V	0	0	+	0		+	0	+	0	SUC +, RAF 0
A. gerencseriae		0	0	V	0	0	+	0		+	0	+	0	SUC +, RAF $+^0$ αMAN +
A. graevenitzii		+	0	V	0	0	0	0		V	+	+	0	
A. israelii		0	0	+	0	0	+	0		+	0	+	+	αMAN 0
A. meyeri		0	0	V	+	0	0	0		+	+	+	$+^0$	
A. naeslundii		0	0	V	0	+	V	0		+	0	+	0	
A. neuii subsp *neuii*		0	+	+	+	0	0	0		+	0	+	+	
A. neuii subsp *anitratus*		0	+	0	+	0	+	0		+	0	+	0	
A. odontolyticus		+	0	+	0	0	V	V		V	0	+	0	
A. radicidentis		+	+	+	0	0	wk	0		+	0	+	0	
A. radingae		0	0	V	+	0	+	+		+	V	+	+	
A. turicensis		0	0	0	0	0	0^+	V		+	0	0	0	
A urogenitalis		+	0	+	0	0	+	0		+	+	+	wk	
A. viscosus		0	+	+	0	0^+	0	0		+	0	V	0	
Arcanob. bernardiae	V	0	0	0	0	0	0	+	0	+	V	0	wk	
Arcanob. haemolyticum	0^+	0	0	0	$+^{rev}$	0	0	+	0^+	+	+	+	0	
Arcanob. pyogenes	+	0	0	0	0	0	0	0	+	+	0	+	0	
Actinobaculum schaalii	+	0	0	0	wk	0	0	0	0	+	0	0	+	
Actinob. urinale	0	0	0	0	.	+	0	0	+	0	0	0	.	PRO +

PIGM = Pigment, CAT = catalase, NO_3 Nitrate reduction D.T., CAMP = CAMP reaction, URE = Urease D.T. ESC = Esculin Hydrolysis D.T., αFUC = Alpha-Fucosidase D.T., αGLU = Alpha-Glucosidase D.T., NAG = Beta-N-Acetylglucosaminidase D.T., ARA = Arabinose D.T., Vanco 5 = Vancomycin 5 µg Neo-S (S ≥ 20 mm, R ≤ 18 mm), Kana 500 = Kanamycin 500 µg Neo-S (S ≥ 10 mm, R < 10 mm), Col = Colistin 10 µg Neo-S (S ≥ 10 mm, R < 10 mm), Metro = Metronidazole 5 µg D.T. (S ≥ 15 mm, R = no zone), PGUA = Beta-Glucuronisase D.T., PRO = Proline Aminopeptidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., Cipro = Ciprofloxacin Neo-S.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Esculin Hydrolysis (Esculin)	*K. pneumoniae* ATCC 13883	*E. coli* ATCC 25922

© Copyright Rosco Diagnostica A/S

References

1) Banton C.E. et al: Abccess caused by vancomycin-resistant Lactobacillus confusus. J.Clin. Microbiol. **29**, 2063-4, 1991.
2) Farmer III J.J. et al: Pyrazinamidase, CR-MOX Agar, Salicin fermentation-Esculin hydrolysis and d-xylose fermentation for identifying pathogenic serotypes of Yersinia enterocolitica. J. Clin. Microbiol. **30**, 2589-94, 1992.
3) Sarkonen N. et al: Phenotypic identification of Actinomyces and related species isolated from human sources. J. Clin. Microbiol. **39**, 3955-61, 2001.
4) Santala A.M. et al: Evaluation of four commercial test systems for identification of Actinomyces and some closely related species. J. Clin. Microbiol. **42**, 418-420, 2004.

© Copyright Rosco Diagnostica A/S

3.11 C-390

REF No. 41611

An antimicrobial agent, 9-chloro-9-(4-diethylaminophenyl)-10- phenylacridan (C-390) has demonstrated exceptional selective properties for ***Pseudomonas aeruginosa*** (1,2,3).

C-390 Diagnostic Tablets contain 40 µg diffusible amount per tablet, and are useful for the identification of *Pseudomonas aeruginosa*. C-390 is packed in cartridges of 50 tablets that may be used with a dispenser.

Procedure
Place one C-390 Diagnostic Tablet on an inoculated plate (Mueller-Hinton Agar) for sensitivity testing. Incubate at 35-37 °C for **18-24 hours**. Read the diameter of the inhibition zone in mm. Iso-sensitest Agar may also be used.

Results

	Semi-confluent growth	Confluent growth (Kirby-Bauer)
***Pseudomonas aeruginosa*:**	zone <12 mm	no zone
Other *Pseudomonas* spp. and non-fermenters:	zone ≥15 mm	≥12 mm

Some strains of *Alcaligenes xylosoxidans* may give small zones of inhibition with C-390 Diagnostic Tablets.

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
C-390 40 µg	*S. maltophilia* ATCC 13637 *E. coli* ATCC 25922 (18-26 mm)	*P. aeruginosa* ATCC 27853 (No zone of inhibition)

References
1) Davis J.R. et al.: "4-h Identification of Pseud. aeruginosa with 9-chloro-9- (4-diethylaminophenyl) -10-phenylacridan". J. Clin. Microbiol. **17**, 1054-1056, 1983.
2) Araj G.F.: "Use of 9-chloro-9-(4-diethylaminophenyl) -10-phenylacridan as a primary medium for recovery of Pseud. aeruginosa from clinical specimens". J. Clin. Microbiol., **20**, 330-333, 1984.
3) Yu P.K.W. et al.: "Comparison of C-390 and cetrimide in the identification of Pseud. aeruginosa". Abstract 624. ICAAC 1985.
4) Casals J.B., Pringler N.: "Identification of Pseudomonas aeruginosa with a C-390 Diagnostic Tablet", 4th European Congress of Clinical Microbiology, Nice, 1989, poster 515.
5) von Graevenitz A. et al.: "Isolation of an unclassified non-fermentative gram-negative rod from a patient on continuous peritoneal dialysis". Eur. J. Clin. Microbiol. Infect. Dis. **12**, 568-570, 1993.
6) Anthony M. et al: Genetic analysis of *Ps. aeruginosa* isolates from the sputa of Australian adult cystic fibrosis patients. J. Clin. Microbiol. **40**, 2772-2778, 2002.

© Copyright Rosco Diagnostica A/S

3.12 CITRATE (CIT)

REF No. 56511

Diagnostic Tablets for testing alcalinization of citrate. Mainly used in the identification of Enterobacteriaceae and non-fermenting gram-negative bacteria.

Procedure
Prepare a dense bacterial suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one Citrate Diagnostic Tablet and close the tube. Incubate at 35-37 °C for 18-24 hours. Positive reactions can sometimes be observed after 4-6 hours incubation.

Reading of the test
Positive reactions: **Red**
Negative reactions: Yellow/orange

Results

Citrate may be used in the differentiation of Enterobacteriaceae.

CIT positive	
Citrobacter spp.	+
Enterobacter spp.	+
Serratia spp.	+
Providencia spp.	+
Klebsiella pneumoniae/oxytoca	+

CIT negative	
E. coli	0
Shigella spp.	0
Edwardsiella spp.	0
Morganella morganii	0
Proteus vulgaris	0^{+}
Yersinia spp.	0

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Citrate (Citrate)	*P. aeruginosa* ATCC 27853	*Proteus vulgaris* ATCC 13315

References
1) Farmer III J.J. et al: Biochemical identification of new species and biogroups of Enterobacteriaceae isolated from clinical specimens. J. Clin. Microbiol. **21**, 46-76, 1985.

© Copyright Rosco Diagnostica A/S

3.13 CYCLOHEXIMIDE (CYC)

REF No. 58921

Cycloheximide (actidione) is a chemical substance which shows activity against several species of fungi. Cycloheximide Diagnostic Tablets contain 15 µg of diffusible amount per tablet. The difference in sensitivity of **Candida species** to cycloheximide may be useful in the identification of these strains.

Procedure
Place one Cycloheximide Diagnostic Tablet on an inoculated plate (Modified Shadomy agar) for sensitivity testing. Incubate at 30-37 °C for **18-24 hours**. Read the diameter of the inhibition zone in mm.

Reading of the tests
Sensitive: **zone ≥ 25 mm** (MIC ≤ 16 µg/ml)
Resistant: zone < 25 mm

Results

The following Candida species are **sensitive**: *C.(Tor.) glabrata*, *C. krusei*, *C. lusitaniae*. Other sensitive fungi are: *Cryptococcus* spp., *Saccharomyces cerevisiae*.

The following Candida species are found **resistant**: *C. albicans*, *C. pseudotropicalis*, *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*. Other resistant fungi are: *Trichosporon* spp. and *Geotrichum candidum*.

Within the resistant strains, we may differentiate between strains showing a) no zone of inhibition and b) a small zone of inhibition (< 25 mm).

a) No zone: *C. albicans*, *C. pseudotropicalis*
b) Small zone: *C. guilliermondii*, *C. parapsilosis*, *C. tropicalis*

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
Cycloheximide 15 µg (Cycloheximide)	*C. krusei* ATCC 6258	*C. albicans* ATCC 64548

References
1) Salkin I.F.: New medium for differentiation of Candida albicans from Candida stellatoidea. J. Clin. Microbiol. **9**, 551-553, 1979.
2) Sobczak H.: A simple disk-diffusion test for differentiation of yeast species. J. Med. Microbiol. **20**, 307-316, 1985.

© Copyright Rosco Diagnostica A/S

3.14 DEFEROXAMINE (DEFRX)

REF No. 59611

Deferoxamine is a siderophore that has been used in the differentiation of coagulase negative staphylococci.

Deferoxamine Diagnostic Tablets contain 250 µg diffusible amount per tablet and are useful for the identification of *Staphylococcus epidermidis* and *Staphylococcus hominis*.

Principle
Deferoxamine is an iron-chelating agent. Most staphylococci need iron in the media for growth. *S. aureus* grows well under conditions of iron restriction, while most coagulase negative staphylococci need certain amounts of iron in the medium. Deferoxamine Diatabs chelates most of the iron around the tablet and consequently particularly *S. epidermidis* and *S. hominis* cannot grow in the vicinity of Deferoxamine Diatabs, resulting in an inhibition zone, while other staphylococci are not affected.

Procedure
Place one Deferoxamine Diagnostic Tablet on an inoculated plate (Mueller-Hinton II or similar) for sensitivity testing. Incubate at 35-37 °C **overnight**. Read the diameter of the inhibition zone.

Please note:
1) Use agar media **without** blood. Blood-agar media are useless for this test (iron-chelating).
2) Measure the zone up to colonies of normal size. Particulary with *S. epidermidis* semi-inhibited colonies are found inside the inhibition zone. They should be disregarded.

Results

1) Staphylococci

	DEFRX Zone of inhibition in mm
Staphylococcus epidermidis	≥ 16 mm (S)
Staphylococcus. hominis	≥ 16 mm (S)
Staphylococcus lutrae	≥ 16 mm (S)
Other staphylococci *	≤ 14 mm (R)

* Other staphylococci includes: *S. aureus*, *S. hæmolyticus*, *S. warneri*, *S. simulans*, *S. capitis*, *S. lugdunensis*, *S. schleiferi*, *S. auricularis*, *S. saprophyticus*, *S. xylosus*, *S. cohnii*.
DEFRX = Deferoxamine D.T.

2) Coagulase negative staphylococci, human (Powerful discriminating tests)

	DEFRX	**Fosfo**	**Novo**	**PYR (1h)**	**ODC**
S. epidermidis	S (≥16 mm)	S (≥30 mm)	S (≥14 mm)	0	0
S. hominis	S	R (<28 mm)	S	0	0
S. simulans	R (≤14 mm)	S	S	+	0 , HCF 0
S. haemolyticus	R	R	S	+	0
S. schleiferi	R	S	S	+	0 , HCF +
S. lugdunensis	R	S	S	+	+
S. saprophlyticus	R	R	R (≤13 mm)	0	0
S. cohnii	R	S	R	0	0
S. xylosus	R	S	R	+	0
S. warneri	R	R	S	0	0
S. capitis	R	R (no zone)	S	0	0

DEFRX = Deferoxamine D.T., Fosfo = Fosfomycin Neo-S, Novo = Novobiocin 5 µg D.T., ODC = Ornithine Decarboxylase D.T, PYR(1h) = Pyrrolidonyl Aminopeptidase D.T. (Incubation 1 hour), HCF =Human Clumping Factor.

© Copyright Rosco Diagnostica A/S

3) CNS mastitis staphylococci

	DEFRX	Novo	Fosfo
S. lugdunensis	R (≤14mm)	S (≥14 mm)	S (≥30 mm)
S. simulans	R	S	S
S. warneri	R	S	R
S. haemolyticus	R	S	R (<28mm)
S. epidermidis	S (≥16 mm)	S	S
S. hominis	S	S	R
CNS (Novo R)	R	R (<13mm)	V

DEFRX = Deferoxamine D.T., Novo = Novobiocin 5 µg D.T., Fosfo = Fosfomycin Neo-S.

4) Coagulase positive staphylococci

	DEFRX	Poly	VP (4h)	MAL	TRE	PYR (1h)
S. aureus	R (≤14mm)	R (≤12mm)	+	+	+	0 wk
S. intermedius	R	S (≥14 mm)	0	0w	+	+
S. pseudintermedius	R	S (≥14 mm)	+	+	+	+
S. schleiferi (coagulans)	R	S	+	0	0	+
S. hyicus	R	S	0	0	+	·
S. delphini	R	S	0	+	0	·
S. lutrae	S (≥16 mm)	S	0	+	+	·

DEFRX = Deferoxamine D.T., Poly = Polymyxin Neo-S, VP(4h) = Voger Proskauer D.T. (4 hours incubation), MAL = Maltose D.T., TRE = Trehalose D.T., PYR (1h) = Pyrrolidonyl Aminopeptidase D.T. (1 h incubation).

5) Ralstonia/Cupriavidus (7,8) (Wautersia) Most strains are: CAT +, Oxi +, PYR +, TRYP 0.

	COL10	DEFRX	MAN	Alk P	URE
Ralstonia pickettii	R	S	0	0	$+^{0}$
R. mannitolilytica	R	S	+	0	+
Cupriavidus insidiosus	R	R	0	0	wk
Cupriavidus gilardii	S	R	0	+	0
Cupriavidus pauculus (IVc-2)	S	R	0	+	$+^{R}$

COL10 = Colistin 10 µg (S ≥13 mm, R ≤ 10 mm), DEFRX = Deferoxamine D.T., (S ≥16 mm), MAN = Mannitol D.T., Alk P = Alkaline Phosphatase D.T., URE = Urease D.T., $+^{R}$ = rapid positive, CAT = catalase, OXI = Oxidase, PYR = Pyrrolidonyl Aminopeptidase D.T., TRYP = Trypsin D.T.

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
Deferoxamine 250 µg (Deferoxamine mesylate)	*S. epidermidis* ATCC 12228	*S. aureus* ATCC 25923

References

1) Lindsay J.A., Riley T.V.: Susceptibility to desferrioxamine: a new test for the identification of Staphylococcus epidermidis. J. Med. Microbiol., **35**, 45-48, 1991.
2) Devriese L.A. et al: A simple identification scheme for coagulase negative staphylococci from bovine mastitis". Research in Vet. Science **57**, 240-4, 1994.
3) Mulder J.G.: A simple and inexpensive method for the identification of Staph. epidermidis and Staph. hominis. Eur. J. Clin. Microbiol. Infect. Dis. **14,** 1052-6, 1995.
4) Foster G. et al: Staph. lutrae sp. nov. of new coagulase-positive species isolated from otters. Intl. J. Syst. Bacteriol. **47**, 724-6,1997.
5) Kahlmeter G. et al: S.lugdunensis - orsakar inte bara endokardit, 1998.
6) Nuttall N.: Identification of clinically significant coagulase negative staphylococci. Workshop 4th South Pacific Congress 9-13 October 1995.
7) De Baere T. et al: Classification of Ralstonia pickettii biovar 31 "thomasii" strains and of new isolates related to nosocomial recurrent meningitis as Ralstonia mannitolilytica sp. nov. IJSEM **51**, 547-558, 2001.
8) Vay C. et al: Bacteremia due to Cupriavidus pauculus (formerly CDC group IVc-2) in a haemodyalisis patient. Clin. Microbiol. Newsletter, **29**, 30-32, 2007.

© Copyright Rosco Diagnostica A/S

3.15 DOUBLE TEST Diatabs

Double Test Diatabs permit performing **two tests** using **one tablet**.

Double test reactions are read as follows:

After incubation for **4 hours** or (**18-24 hours**) at 35-37°C
a) the first reaction is read **without reagent** addition providing the first test result, and
b) in the same tube the second reaction is read **after reagent addition**, providing the second test result.

The following Double Test Diatabs are currently available:

LDC/Indole	Enterobacteriaceae
ODC/Indole	Enterobacteriaceae
PGUA/Indole	*E. coli*
Urease/Indole	Enterobacteriaceae, Non-Fermenters
Urease/TDA	Enterobacteriaceae

The use of simplified rapid testing results in up to 75 % reduction in cost of reagents and technologist time, with a decrease in time to reporting.

© Copyright Rosco Diagnostica A/S

3.15.1 LDC / INDOLE (LDC/IND)

REF No. 58411

Double Test tablet for Lysine decarboxylase (LDC) and Indole test, mainly for use in the identification of **Enterobacteriaceae**.

Procedure
Prepare a dense suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a small tube. Add one diagnostic tablet and **3 drops of paraffin oil** and close the tube. The oil overlayer provides anaerobic conditions necessary to avoid false positive reactions for the lysine decarboxylase test. Incubate at 35-37 °C for **3-4 hours** (or **up to 24 hours**).

Reading of the tests

Lysine decarboxylase (LDC)
NB! The Lysine decarboxylase test must be read before adding reagent for the Indole test.

Positive reaction: **Blue/violet**
Negative reaction: Yellow, green, grey

After **overnight** incubation **only strong blue or violet** is positive!

Indole
After reading the LDC test add **3 drops of Kovacs' reagent** (92031), shake gently and wait for 3 minutes. Look only at the **colour of the surface layer**.

Positive reaction: **Red** (surface layer)
Negative reaction: Yellow

Results

1) Screening for Salmonella/Shigella (1). LOUIS Test (3 hours)

LDC	ONPG	URE	IND	Possible ID	Step 1	Step 2
+	+	0	+	*E. coli*	Discard	
+	0	0	+			
0	0	+	+	*Proteus* spp.	Discard	
0	0	+	0	Morganella		
+	0	0	0	Salmonella	Confirm by serology	Neg. Discard
0	0	0	0	*Shigella* spp. (LDC neg. Salmonella)	Confirm by serology	Neg. Discard
0	0	0	+	*Shigella* spp.	Confirm by serology	Neg. Discard
0	+	0	0	*Shigella sonnei* or *Sh. dysent. I*	Confirm by serology	Neg. Discard

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
LDC/Indole (L-Lysine, L-Tryptophane)	*E. coli* ATCC 25922 (LDC pos., IND pos.)	*Proteus vulgaris* ATCC 13315 (LDC neg., IND pos.) *K. pneumoniae* ATCC 13883 (LDC pos., IND neg.)

References
1) Wilson G.: Rapid and economical method for biochemical screening of stool isolates for Salmonella and Shigella species. J.Clin. Microbiol. **42**, 4821-3, 2004.

© Copyright Rosco Diagnostica A/S

3.15.2 ODC / INDOLE (ODC/IND)

REF No. non-stock
(59121)

Double Test tablet for Ornithine decarboxylase (ODC) and Indole test, mainly for use in the identification of Enterobacteriaceae.

Procedure
Prepare a dense suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a small tube. Add one diagnostic tablet and **3 drops of paraffin oil** and close the tube. The oil overlayer provides anaerobic conditions necessary to avoid false positive reactions for the ornithine decarboxylase test. Incubate at 35-37 °C for **3-4 hours** (or **up to 24 hours**).

Reading of the tests

Ornithine decarboxylase (ODC)
NB! The Ornithine decarboxylase test must be read before adding reagent for the Indole test.

Positive reaction: **Blue/violet**
Negative reaction: Yellow, green, grey

After **overnight** incubation **only strong blue or violet** is positive!

Indole
After reading the ODC test add **3 drops of Kovacs' reagent** (92031), shake gently and wait for 3 minutes or more. Look only at the **colour of the surface layer**.

Positive reaction: **Red** (surface layer)
Negative reaction: Yellow

Results

1) Differentiation of *Citrobacter* spp.

	ODC	IND	DUL	ESC	MALON	MEL	RAF	Remarks
C. freundii	0	0	0^+	0	0	+	$+^0$	
C. koseri	+	+	V	0^+	+	0	0	ADON $+^0$
C. amalonaticus	+	+	0	0^+	0	0	0	ß-XYL V
C. braaki	+	0	V	0	0	+	0	
C. farmeri	+	+	0	0^+	0	+	+	
C. gillenii	0	0	0	V	+	$+^0$	0^+	
C. murliniae	0	+	+	V	0	V	0^+	
C. sedlakii	+	+	+	+	+	+	0	
C. werkmanii	0	0	0	0	V	0	0	
C. youngae	0^+	0	$+^0$	0	0^+	0	0	

ODC/IND = ODC/Indole D.T., DUL = Dulcitol D.T., ESC = Esculin Hydrolysis D.T., MALON = Malonate, MEL = Melibiose D.T., RAF = Raffinose D.T., ADON = Adonitol D.T., β-XYL = Beta-Xylosidase D.T.

© Copyright Rosco Diagnostica A/S

2) Differentiation of biotypes of *H. influenzae* (4)

	ODC	IND	URE
Biotype I	+	+	+
Biotype II	0	+	+
Biotype III	0	0	+
Biotype IV (*H. quentini)*	+	0	+
Biotype V	+	+	0
Biotype VI	+	0	0
Biotype VII	0	+	0
Biotype VIII	0	0	0

ODC/IND = ODC/Indole D.T., URE = Urease D.T.

3) Differentiation of most common *Vibrio* spp. (human interest)

Most *Vibrio* spp. are OXI +, O/129 S, NO_3 +. Inoculum on 2.5 % NaCl solution, incubation at 30 °C.

	IND	ADH	LDC	ODC	ONPG	ARA	MAN	PRO	VP	COL	Remarks
Vibrio cholerae	+	0	+	+	+	0	+	0^+	75	R	TTR 0
Vibrio mimicus	+	0	+	+	$+^0$	0	+	0	0	S^R	
Vibrio metschnikovii	20	60	35	0	50	0	+	·	+	S	OXI 0, NO_3 0
Vibrio hollisae	+	0	0	0	0	+	0	0	0	S	NAG 0^+, PYR +
Photobacterium (Vibrio) damsela	0	$+^0$	50	0	0	0	0	0	$+^0$	S^R	PYR +
Vibrio fluvialis/ V. furnisii	0^+	$+^0$	0	0	40	$+^0$	+	+	0	S	
Vibrio alginolyticus	$+^0$	0	+	50	0	0	+	+	+	R^S	TRYP +, TTR +
Vibrio parahaemolyticus	+	0	+	+	0^+	80	+	+	0	R^S	TRYP +, TTR +
V. vulnificus bio 1	+	0	+	$+^0$	V	0	$+^0$	+	0	R	SORB 0
V. vulnificus bio 2	V	0	+	0	V	0	0	·	0	R	SORB +
V. vulnificus bio 3	+	0	+	+	V	0	0	·	0	R	SORB 0
Vibrio harveyi	+	0	+	0	0	0	50	·	50	R	

ADH = Arginine Dihydrolase D.T., LDC = Lysine Decarboxylase D.T., ARA = Arabinose D.T. , MAN = Mannitol D.T., PRO = Proline Aminopeptidase D.T., VP = Voges Proskauer D.T., COL = Colistin 10 µg Neo-S (S ≥ 13 mm, R = ≤ 10 mm), TTR Tetrathionate Reductase D.T., NO_3 = Nitrate Reduction D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., TRYP = Trypsin D.T., O/129 D.T. (S ≥ 16 mm, R < 16 mm).

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
ODC/Indole (L-Ornithine, L-Tryptophane)	*E. coli* ATCC 25922 (ODC. pos, IND pos.)	*K. pneumoniae* ATCC 13883 (ODC neg., IND neg.)

References

1) Brenner D.J. et al: Classification of Citrobacteria by DNA hybridization: Designation of C. farmeri sp. nov., C. youngae sp. nov., C. braakii sp. nov., C. werkmanii sp. nov., C. sedlakii sp. nov. and 3 unnamed Citrobacter genomospecies. Intl. J. Syst. Bacteriol. **43**, 645-658, 1993.
2) Janda M.J. et al: Biochemical identification of Citrobacteria in the clinical laboratory. J. Clin. Microbiol. **32**, 1850-4, 1994.
3) Brenner D.J. et al: Biochemical identification of Citrobacter species defined by DNA hybridization and description of Citrobacter gillenii sp. nov. and C. murliniae sp. nov.. J. Clin. Microbiol. **37**, 2619-24, 1999.
4) Campos J.M.: Haemophilus. Manual of Clinical Microbiology 6th ed. chapter **45**, 557-565, 1995.
5) Vibrio Key differential Tests. Manual of Clinical Microbiology 8th ed., 707-712, 2003.
6) Mak G.C. et al: Reduced levofloxacin susceptibility and tetracycline resistance in a clinical isolate of Haemophilus quentini identified by 16S or RNA sequencing. J. Clin. Microbiol. **43**, 5391-5392, 2005.

© Copyright Rosco Diagnostica A/S

3.15.3 PGUA / INDOLE (PGUA/IND)

REF No. 59011

Double Test tablet for Beta Glucuronidase (PGUA) and Indole test, mainly for use in the identification of ***Escherichia coli*** e.g. from urinary tract infections.

Approx. 94 % of *E. coli* are positive for PGUA and approx. 99 % are positive for Indole.

The use of simplified identification systems saves laboratory resources, results in up to 75% reduction in cost of reagents and technologist time with a reduction in time to reporting (4).

Procedure
Prepare a dense suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a small tube. Add one diagnostic tablet and close the tube. Incubate at 35-37 °C for **3-4 hours** (or **up to 24 hours**).

Reading of the tests

Beta Glucuronidase (PGUA)
NB! The Beta Glucuronidase test must be read before adding reagent for the Indole test.

Positive reaction: **Yellow**
Negative reaction: Colourless

Indole
After reading the PGUA test add **3 drops of Kovacs' reagent** (92031), shake gently and wait for 3 minutes. Look only at the **colour of the surface layer**.

Positive reaction: **Red** (surface layer)
Negative reaction: Yellow

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
PGUA/Indole (p-Nitrophenyl-β-D-Glucuronic acid, L-Tryptophane)	*E. coli* ATCC 25922 (PGUA pos., IND pos.) *Proteus vulgaris* ATCC 13315 (PGUA neg., IND pos.)	*Enterobacter cloacae* ATCC 13047 (PGUA neg., IND neg.)

References
1) Iritani B. et al: Evaluation of a rapid tube assay for presumptive identification of E. coli from veterinary specimens". J. Clin. Microbiol. **26**, 564-6, 1988.
2) Casals J.B., Pringler N.: Rapid Identification of E. coli with a Double Test Tablet: Beta Glucuronidase (PGUA)/Indole. 4th European Congress of Clinical Microbiology, Nice, 1989, poster 514.
3) Domínguez A., Alcaide F., Pulido A., Ayats J., Pérez J.L., Martín R.: Use of a Commercial Double-Test Tablet (Rosco PGUA/Indole) for Screening of Escherichia coli. Diagn. Microbiol. Infect. Dis. **15**, 291-294, 1992.
4) York M.K. et al.: Multilaboratory validation of rapid spot tests for identification of E.coli. J. Clin. Microbiol. **38**, 3394-8, 2000.

© Copyright Rosco Diagnostica A/S

3.15.4 UREASE / INDOLE (URE/IND)

REF No. 57611

Double Test tablet for the Urease test and the Indole test; both tests are commonly used in identification of e.g. **Enterobacteriaceae** and **non- fermenting gram-negative bacteria**.

Procedure
Prepare a dense suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one diagnostic tablet and close the tube. Add. 3 drops of paraffin oil and incubate at 35-37 °C for **4 hours** (or **18-24 hours**). For "non-fermenters" overnight incubation is recommended.

Reading of tests

Urease
NB! The urease test must be read before adding reagent for the Indole test.

Positive reaction: **Red/purple**
Negative reaction: Yellow

After overnight incubation only strong red/purple is positive!

Indole
After reading the Urease test add **3 drops of Kovacs' reagent** (92031), shake gently and wait for 3 minutes. Look only at the **colour of the surface layer**.

Positive reaction: **Red** (surface layer)
Negative reaction: Yellow/orange

Results

1) Differentiation of *Actinobacillus* spp. from *Pasteurella* spp./*Mannheimia* spp. (CAT +, OXI +)

	URE	IND	α-GLU	SUC	Remarks
Actinobacillus spp.	+	0	V	+	
Pasteurella spp.	0^+	+	+	+	
Mannheimia spp.	0	0		+	
Haemophilus spp.	V	V	0	0	Factor X/V +

α-GLU = Alpha-Glucosidase D.T. SUC = Sucrose D.T., Factor X D.T., Factor V D.T.

2) Differentiation of *Pasteurella* spp. (human interest)

Most strains are: OXI +, CAT +, NO_3 +, ADH 0, URE 0, ESC 0, O/129 S, MOT 0.

	CAT	IND	URE	ODC	ONPG	MAL	TRE	MAN	SOR	Remarks
"P. caballi"	0	0	0	+	+	$+^0$	0	+	0	
P. canis bio 1	+	+	0	+	0	0	$+^0$	0	0	
P. canis bio 2	+	0	0	+	0	0	+	0	0	
P. dagmatis	+	+	+	0	0	+	+	0	0	
P. langaaensis	0	0	0	0	+	0	0	+	0	
P. multocida ssp. *multocida*	+	$+^0$	0	$+^0$	0	0^+	$+^0$	$+^0$	+	DUL 0, α-GLU +
P. multocida ssp. *septica*	+	+	0	$+^0$	0	0^+	+	+	0	DUL 0, α-GLU +
P. multocida ssp. *gallicida*	+	+	0	+	0	0^+	0	+	+	DUL +, α-GLU 0
Taxon 45 Bisgaard	+	+	0	+	0	0^+	0	0	+	α-GLU 0^+, SUC 0
P. stomatis	+	$+^0$	0	+	0	0	+	0	0	
Gallibacterium anatis	+	0	0	0	+	V	$+^0$	+	V	α-GLU +
Avibacterium avium	$+^0$	0	0	0	0	0	+	0	0	
Avibacterium gallinarum		0	0	0	0	+	+	0	0^+	

CAT = catalase, URE/IND = Urease/Indole D.T., ODC = Ornithine Decarboxylase D.T., MAL = Maltose D.T., TRE = Trehalose D.T., MAN = Mannitol D.T., SOR = Sorbitol D.T., DUL = Dulcitol D.T., α-GLU = Alpha-Glucosidase.

© Copyright Rosco Diagnostica A/S

3) Differentiation of *clostridia* producing neurotoxins (Gel +)

	IND	LEC	ESC	NO_3	URE
C. tetani	75	0	0	0	0
C. botulinum type B	0	0	+	0	+
Clostridium spp. RKD	+	+	0	+	+

Gel = gelatinase, LEC = lecithinase, ESC = Esculin Hydrolysis D.T., NO_3 = Nitrate Reduction D.T., URE/IND = Urease/Indole D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Urease/Indole (Urea, L-Tryptophane)	*Morganella morganii* ATCC 25830 (URE pos., IND pos.) *K. pneumoniae* ATCC 13883 (URE pos., IND neg.)	*E. coli* ATCC 25922 (URE neg., IND pos.)

References

1) Ashhurst-Smith C. et al.: Actinobacillus equuli septicemia: an unusual zoonotic infection. J. Clin. Microbiol. **36**, 2789-90, 1998.
2) Euzéby J.P. Dictionnaire de bacteriologie veterinaire. March 2001.
3) Gerards S.H. et al: Pasteurella multocida ssp. multocida and P. maltocida ssp. septica. Differentiation by PCR fingerprinting and α-glucosidase activity. J. Clin. Microbiol. **39**, 2558-64, 2001.
4) Aparma Dixit et al: Characterization of Clostridium spp. RKD producing botulinum–like neurotoxin. System. Appl. Microbiol. **28**, 405-414, 2005.

© Copyright Rosco Diagnostica A/S

3.15.5 UREASE / TDA (URE/TDA)

REF No. 57911

Double Test tablet for the Urease test and the Tryptophane deaminase test (TDA). The tablet is mainly used in identification of **Enterobacteriaceae** and is especially useful in differentiation of the **Proteus-Morganella-Providencia-group** (TDA positive) from the rest of the family.

Procedure
Prepare a dense suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a small tube. Add one diagnostic tablet and close the tube. Incubate at 35-37 °C for **3-4 hours** (or **18-24 hours**).

Reading of the tests

Urease
NB! The Urease test must be read before adding reagent for the Tryptophane deaminase test.

Positive reaction: **Red/purple**
Negative reaction: Yellow

After overnight incubation only strong red/purple is positive!

Tryptophane deaminase (TDA)
After reading the Urease test add **2 drops of Ferric Chloride 10% solution** and read within 5 minutes.

Positive reaction: **Red/brown**
Negative reaction: Yellow/orange

Indole-positive strains may produce an orange colour due to indole production. This is a negative reaction.

Results

	URE	TDA
Proteus spp.	$+^R$	+
Morganella spp.	$+^R$	+
Providencia spp.	V	+
Other Enterobacteriaceae	V	0

$+^R$ = rapid positive reaction

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Urease/TDA (Urea, L-Tryptophane)	*Proteus vulgaris* ATCC 13315 (URE pos., TDA pos.) *K. pneumoniae* ATCC 13883 (URE pos., TDA neg.)	*E. coli* ATCC 25922 (URE neg., TDA neg.)

© Copyright Rosco Diagnostica A/S

3.16 GENTAMICIN 250 µg (GN250), KANAMYCIN 500 µg (KA500), STREPTOMYCIN 500 µg (ST500) Neo-Sensitabs

REF No. 43012
REF No. 43112
REF No. 44712

High content tablets for detection of **high level resistance (HLR) towards the aminoglycosides** in enterococci and streptococci.

Kanamycin 500 µg is also useful in the presumptive identification of anaerobes.

In several countries approx. 50 % of *E. faecalis* isolates are highly resistant to streptomycin (MIC >2000 µg/ml) and HLR to gentamicin is increasing rapidly. Low content discs and automatized methods have difficulties in detecting this kind of resistance.

Procedure
The media recommended are: Mueller-Hinton II **without blood for enterococci** and M-H II with 5% blood for streptococci. The inoculum is standardized as for routine sensitivity testing (0.5 McFarland).

Reading of the tests
Zone diametres and the corresponding MIC values are as follows:

	Zone diameter high level resistant	**Equivalent MIC**
Gentamicin 250 µg	< 14 mm (HLR)	> 500 µg/ml
Kanamycin 500 µg	< 14 mm (HLR)	> 1000 µg/ml
Streptomycin 500 µg	< 14 mm (HLR)	> 1000 µg/ml

In general we may conclude:

a) If a strain shows HLR to Streptomycin: this aminoglycoside will not show synergistic killing in combination with a penicillin (or vancomycin).

b) If a strain shows HLR to Kanamycin: this aminoglycoside and amikacin cannot be used.

c) If a strain shows HLR to Gentamicin: then the strain is HLR to all aminoglycosides, except streptomycin. Streptomtycin might be useful, if the strain does not show HLR to streptomycin.

E. faecium shows intrinsic resistance towards kanamycin, tobramycin and netilmicin due to the production of the enzyme AAC (6'). Consequently there is no synergy with beta-lactams.

Quality Control

NEO-SENSITABS	Potency	Code	***E. faecalis* ATCC 51299**	***E. faecalis* ATCC 29212**
Gentamicin	250 µg	GN 250	no zone (R)	17-23
Streptomycin	500 µg	ST500	no zone (R)	-

MH-agar, inoculum McF 0.5, incubation 35 °C 16-18 hours.

© Copyright Rosco Diagnostica A/S

References

1) Amsterdam D.: Simple detection of high level resistance of Enterococcus faecalis to aminoglycosides. An alternative to synergy testing. The Antimicrobic Newsletter **5**, 36-38, 1988.
2) Spiegel C.A.: Laboratory Detection of High-Level Aminoglycoside Aminocyclitol Resistance in Enterococcus spp. J. Clin. Microbiol. **26**, 2270-2274, 1988.
3) Huycke M.M. et al: Bacteremia Caused by Hemolytic, High-Level Gentamicin- Resistant Enterococcus faecalis. A.A.C. **35**, 1626-1634, 1991.
4) Sahm D.F. et al: Detection of High-Level Aminoglycoside Resistance in Enterococci Other Than Enterococcus faecalis. J. Clin. Microbiol. **29**, 2595-2598, 1991.
5) Torres C. et al: Detection of aminoglycoside-penicillin synergy against Enterococcus faecium using high control aminoglycoside disks. Eur. J. Clin. Microbiol. Infect. Dis. **14**, 878-82, 1995.

© Copyright Rosco Diagnostica A/S

3.17 FACTOR X, V, and X+V

REF No. 42511
REF No. 42611
REF No. 42711

Contain growth factors for the differentiation of ***Haemophilus*** **spp.: Hemin** (X-Factor) and **NAD** (V-Factor).

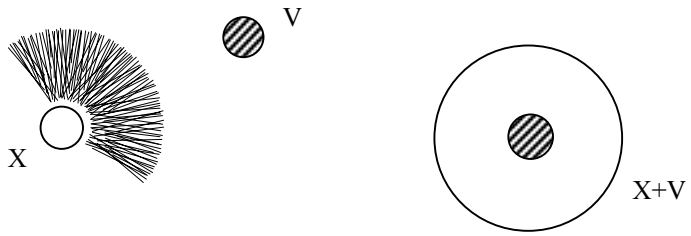
Principle of the Test
Haemophilus influenzae requires both X-Factor and V-Factor for growth, while ***Haemophilus parainfluenzae*** requires V-Factor only. Growth around the diagnostic tablets (and not on the rest of the plate) is taken as evidence of requirement for either growth factor alone or both factors together.

Procedure
Make a suspension in saline (approx. 0.5 McFarland) of colonies from an agar plate and swab the suspension on a medium free of the two growth factors (e.g. TSA agar). Place the diagnostic tablets containing X-, V-, and X+V-Factors onto the agar; Factor X and Factor V at a distance of approx. 2 cm from each other and Factor X+V somewhat further away from these. Incubate the plate in 5-10% CO_2 at 35-37 °C for **18-24 hours**.

Reading of the Test

a) ***Haemophilus influenzae***
Growth is seen only around the Factor X+V tablet and **between** the Factor X and Factor V tablets (Fig. 1).

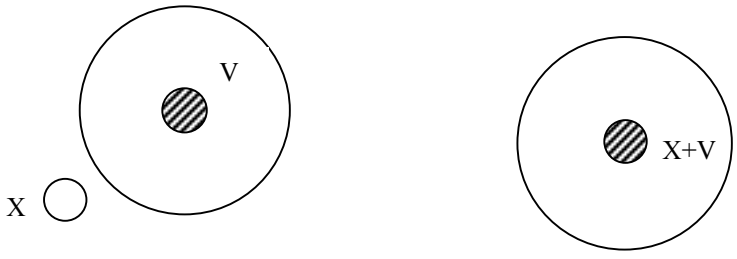
Fig. 1



The area of growth between the Factor X and the Factor V tablets is **closer to the Factor X tablet** due to higher diffusability of V-Factor than X- Factor, giving a semicircle of growth around the X-Factor tablet. *Haemophilus influenzae* strains with very small V-Factor requirements (0.04 mg/liter or similar) may give a **full circle** of growth around the Factor X tablet.

b) ***Haemophilus parainfluenzae***
Growth is seen only around the Factor V and the Factor X+V tablets (Fig. 2).

Fig. 2



The growth zones around Factor X+V are considerably larger than those seen for *Haemophilus influenzae* due to higher diffusability of Factor V compared to Factor X.

© Copyright Rosco Diagnostica A/S

Choice of medium
The medium should be tested with known cultures of *H. influenzae* and *H. parainfluenzae* to make sure it is adequate for the test avoiding the following problems:

a) The medium lacks adequate amounts of other nutrients essential for growth of *Haemophilus* spp.
TSA-agar (e.g. BBL) has been recommended for the test allowing growth of more strains than the less nutrious Mueller-Hinton agar (Doern & Chapin, 1984). Other media may be used, but must be checked for content of X-and V-Factors (see b) and c)).

b) The medium contains hemin (X-Factor)
Haemophilus influenzae will show the reaction of a strain requiring only V-Factor and can be misidentified as *Haemophilus parainfluenzae*. Similar reactions can be seen as a result of carry-over from chocolate agar when preparing the inoculum for the test. Check with known *H. influenzae* strains to assure there is no growth around the Factor V tablet.

c) The medium contains NAD (V-Factor)
Haemophilus influenzae requires only small amounts of V-Factor (approx. 0.04 - 0.2 mg/liter (Evans et al., 1974)), and some media contain sufficient amounts for growth (e.g. from yeast extract (CASO-Agar Merck No. 5458)).
On these media *H. influenzae* gives the pattern of a strain requiring only X-Factor - growth around Factor X and Factor X+V tablets with growth zones of equal size. Small contents of V-Factor will not usually interfere with the reaction of *H. parainfluenzae* as this species requires considerably higher concentrations of V-Factor (approx. 1-5 mg/liter (Evans et al., 1974)).
These media may be used for the test if growth around the Factor X tablet is disregarded. The growth pattern around the Factor V and Factor X+V tablets will be correct.

Quality Control

DIATABS (Active ingredients)	
Factor V (b-Nicotinamide adenine dinucleotide sodium) **Factor X** (Hemin chloride) **Factor X + V**	*H. influenzae* ATCC 49247 *H. parainfluenzae* ATCC 7901

References
1) Doern G.V., Chapin K.C: Laboratory Identification of Haemophilus influenzae: Effects of Basal Media on the Results of the Satellitism Test and Evaluation of the Rap ID NH System. J. Clin. Microbiol. **20**, 599- 601, 1984.
2) Evans N.M., Bell S.M., Smith D.D.: New Satellitism Test for Isolation and Identifica-tion of Haemophilus influenzae and Haemophilus parainfluenzae in Sputum. J. Clin. Microbiol. **1**, 89-95, 1975.
3) Evans N.M., Smith D.D., Wicken A.J.: Haemin and nicotinamide adenine dinucleotide requirements of Haemophilus influenzae and Haemophilus parainfluenzae. J. Med. Microbiol. **7**, 359-365, 1974.
4) Kilian M., Sørensen I., Frederiksen W.: Biochemical characteristics of 130 recent isolates from Haem. influenzae meningitis. J. Clin. Microbiol. **9**, 409-412, 1979.
5) Santanam, P.: A Modified Method for Differentiation of Haemophilus influenzae from Haemophilus parainfluenzae. Eur. J. Clin. Microbiol. **3**, 150-151, 1984.
6) Quentin R., Musser J.M., Mellouet M., Sizaret P.-Y., Selander R.K., Goudeau A.: Typing of Urogenital, Maternal, and Neonatal Isolates of Haemophilus influenzae and Haemophilus para-influenzae in Correlation with Clinical Source of Isolation and Evidence for a Genital Specifity of H. influenzae Biotype IV. J. Clin. Microbiol. **27**, 2286-2294, 1989.

© Copyright Rosco Diagnostica A/S

3.18 FOSFOMYCIN 70 µg (FOSFO) Neo-Sensitabs

REF No. 74212

We have been using Fosfomycin 70 µg Neo-Sensitabs for a long time in our laboratory as an aid in the identification of staphylococci. We find, in accordance with Iwantscheff (1988), that the staphylococci may be divided into three groups:

a) strains resistant to fosfomycin (*S. capitis*),
b) strains with intermediate sensitivity, and
c) the most sensitive strains.

The degree of sensitivity to fosfomycin differs for some species that are otherwise closely related, e.g. *S. saprophyticus* is considerably more resistant than the other novobiocin resistant species, *S. xylosus* and *S. cohnii*.

Procedure
Sensitivity testing is performed on Mueller-Hinton II Agar with an inoculum equivalent to McFarland 0.5. Incubation at 35-37 °C **overnight**.

Results

1) Human staphylococci

	FOSFO
a) *S. capitis* *S. capitis* ssp. ureolyticus *S. caprae*	no zone
b) *S. hominis*, *S. haemolyticus*, *S. warneri*, *S. saprophyticus*	small zone < 28 mm
c) *S. aureus*, *S. epidermidis*, *S. lugdunensis*, *S. schleiferi*, *S. xylosus*, *S. cohnii*, *S. cohnii* ssp. urealyticum, *S. simulans**	zone > 30 mm

* *S. simulans* show growth of resistant colonies inside the inhibition zone (≥ 40 mm).

2) Coagulase negative mastitis staphylococci

	NOVO5	**DEFRX**	**FOSFO**	**Remarks**
S. hyicus	S (≥14 mm)	R (≤14 mm)	S (≥30 mm)	
S. chromogenes	S	R	S	
S. simulans	S	R	S	
S. warneri	S	R	R (≤28 mm),	URE +
S. haemolyticus	S	R	R	URE 0
S. epidermidis	S	S (≥16 mm)	S	
S. hominis	S	S	R	
CNS Novo R	R (<13 mm)	R	V	

NOVO5 = Novobiocin 5 µg Neo-S, DEFRX = Deferoxamine D.T., FOSFO = Fosfomycin 70 µg Neo-S.

3) Corynebacteria and Listeria are resistant to fosfomycin, therefore Fosfomycin 70 µg Neo-Sensitabs may be used on blood agar plates for isolation/screening of diphtheroids (growth near the edge of the tablet).

© Copyright Rosco Diagnostica A/S

References

1) Iwantscheff A.: In-vitro activity of fosfomycin against different Staphylococci species. J. Antimicrob. Chemother. **21**, 379-381, 1988.
2) Devriese L.A. et al: A simple identification scheme for coagulase negative staphylococci from bovine mastitis. Research in Vet. Science **57**, 240-4, 1994.
3) Foster G. et al: Staph. lutrae sp. nov., a new coagulase positive species isolated from otters. Intl. J. Syst. Bacteriol. **47**, 724-6, 1997.
4) Leung M.J.: Colony variation in Staphylococcus lugdunensis. J. Clin. Microbiol. **36**, 3096-8, 1998.
5) von Gravenitz A. et al: Coryneform bacteria in throat cultures of healthy individuals. J. Clin. Microbiol. **36**, 2087-8, 1998.

© Copyright Rosco Diagnostica A/S

3.19 FURAZOLIDONE 50 µg (FURAZ) MUPIROCIN 10 µg (MUPIR) Neo-Sensitabs

REF No. 74412
REF No. 75712

Furazolidone and Mupirocin are useful in the differentiation of **staphylococci** (sensitive) from **micrococci** (resistant). Besides, they are useful in the differentiation of enterococci and some coryneform bacteria.

Procedure
Sensitivity testing of staphylococci or micrococci is performed on Mueller- Hinton II Agar without blood with an inoculum equivalent to McFarland 0.5 using Furazolidone 50 µg Neo-Sensitabs and Mupirocin 10 µg Neo-Sensitabs. Strains that cannot grow on this agar may be tested on Mueller-Hinton II agar with added 5 % blood. Incubate at 35-37 °C **overnight**. If only one test is used, we recommend Furazolidone 50 µg Neo-Sensitabs.

Results

1) Differentiation of staphylococci from micrococci/kitococcus:

1a)	**FURAZ** and **MUPIR**	
Sensitive: (S)	≥ 16 mm:	**staphylococci**
Resistant: (R)	< 16 mm:	**micrococci**

Above interpretation is also valid for semi-confluent growth on Iso-Sensitest, DST, PDM II and Danish Blood Agar.

1b)	**FURAZ**	**OXA**	**ADH**
Staphylococcus spp.	S	V	V
Micrococcus spp.	R	S	0
Kitococcus spp.	R	R	+

2) Differentiation of enterococci

2a)	**MUPIR**	**FURAZ**	**NOVO5**
Enterococcus faecalis	R (NZ)	S	R (<13 mm)
Enterococcus faecium	S	R (NZ)	S (≥14 mm)
Other enterococci	S^R	S	S

2b)	**FURAZ**	**MUPIR**	**MOT**	**PIGM**	**MGP**	**XYL^R**	**α-GAL**	**ARA**	**CLINDA**	**Remarks**
Enterococcus gallinarum	S	S	+	0	+	+	+	+	R	
Enterococcus faecalis	S		0	0	0	0	0	0	R	MAN +
Enterococcus faecium	R (NZ)	S	0	0	0	0	V	+	S	
Enterococcus casseliflavus (flavescens)	S	S	+	+	+	0	+	+	R	
Enterococcus durans	S	R	0	0	·	0	0	0	S	SUC 0
Enterococcus hirae	·	R	0	0	0	·	$+^0$	0	·	MAN 0, SUC $+^0$
Enterococcus caccae	·	·	0	0	+	0	0	0	·	MAN 0, ADH 0, TEL R, NAG +, ß-MAN +,VP +

MUPIR = Mupirocin 10 µg Neo-S, FURAZ = Furazolidone 50 µg Neo-S, NOVO5 = Novobiocin 5 µg Neo-S, MOT = motility, NZ = no zone, PIGM = pigment, MGP = Methyl-α-D-glucopyranoside, R^S = Most strains resistant, XYL^R = Rapid Xylose D.T. (incub. 2 h at 37 °C, McF 3) (9), α-GAL = Alpha-Galactosidase D.T., OXA = Oxacillin Neo-S, ADH = Arginine Dihydrolase D.T., ARA = l-Arabinose, CLINDA = Clindamycin Neo-S, MAN = Mannitol D.T., SUC = Sucrose D.T., TEL = Tellur 500 µg D.T., ß-MAN = Beta-mannosidase, NAG = N-acetyl glucosaminidase D.T., VP = Voges-Proskauer D.T.,

© Copyright Rosco Diagnostica A/S

3) Coryneform bacteria

	FURAZ	O/129	LAP
C. minutissimum	S (zone)	S	+
C. amycolatum	R (no zone)	R	0

O/129 = O/129 150 µg D.T., LAP = Leucine Aminopeptidase.

4) Throat cultures

	BaL	MUPIR	PYR
Arcanobact. haemolyticum	R	R	0
Streptococcus pyogenes (A)	S	S	+
Streptococcus group C/G	R(V)	S	0

BaL = Bacitracin low 0.4 U N.D. (S > 15 mm), MUPIR = Mupirocin 10 µg Neo-S (R = no zone), PYR = Pyrrolidonyl Aminopeptidase D.T.

5) Agents of Zoonotic infections. Differentiation of streptococci, corynebacteria and listeria (11)

	MUPI	VANCO	FOSFO	PYR	PRO	PGUA	H_2S	Haem.
Arconob. pyogenes	R	S	S	+	+	+	0	Beta
Strept. suis	S	S	S	$+^0$	.	$+'^0$	0	Alpha
Erysipel. rhusiopathiae	.	R	S	$+^0$	V	.	+	CAT 0
Streptococci spp.	S	S	S	0^+	.	0^+	.	
Corynebacteria spp.	R	S	R	0^+	$+^0$	0^+	.	α-MAN 0
Listeria spp.	R	S	R	0	0	0	.	α-MAN +

MUPI = Mupirocin Neo-S (S ≥ 16 mm and R < 16 mm), VANCO = Vancomycin Neo-S (S ≥ 16 mm and R < 16 mm), FOSFO = Fosfomycin Neo-S (S ≥ 16 mm and R < 16 mm), PYR = Pyrrolidonyl Aminopeptidase D.T., PRO = Proline Aminopeptidase D.T., PGUA = Beta-Glucuronidase D.T., H_2S = Hydrogen sulphide production. Haem = Haemolysis.

References

1) Ezekiel P.A., Baker J.S.: Evaluation of a furazolidone-peptone Agar and a Furazolidone Disc Diffusion method for differentiating staphylococci from micrococci. Annual Meeting ASM, 1983, Abstract C-367.
2) De la Fuente et al: Comparison of methods for routine separation of coagulase negative staphylococci from micrococci isolated from sheep. Comp. Immunol. Microbiol. Infect. Dis. **9**, 347-353, 1986.
3) Casals J.B., Pringler N.: The value of 3 tests in the identification of staphylococci: Pyrrolidonyl aminopeptidase (PYR) and Susceptibility towards Polymyxins and Furazolidone. Staphylococci Symposium, Society for Applied Bacteriology, Edinburgh, July 1989.
4) Casals J.B., Pringler N.: Identification of staphylococci using a combination of chromogenic substrates and sensitivities towards Furazolidone, Novobiocin and Colistin. Workshop on Pathogenesis of Wound and Biomaterial-Associated Infections, Lund University, 1989.
5) Wegener H.C.: Diagnostic value of phage typing, antibiogramme typing, and plasmid profiling of S. hyicus from piglets with exudative dermatitis. J. Vet. Med. **40**, 13-20, 1993.
6) Chesneau O. et al: Staphylococcus pasteuri sp. nov. Isolated from human, animal and food specimens. Intl. J. Syst. Bacteriol. **43**, 237-44, 1993.
7) Früh M. et al: Use of second-line biochemical and susceptibility tests for the differential identification of coryneform bacteria. Clin. Microbiol. Infect. **4**, 332-8, 1998.
8) Iwen P.C. et al: Evaluation of the revised Microscan dried overnight gram-positive identification panel to identify Enterococcus species. J. Clin.. Microbiol. **37**, 3756-8, 1999.
9) Chen D. K. et al: Evaluation of d-Xylose and 1% Methyl-α-D-glucopyranoside fermentation tests for distinguishing Ent. gallinarum from Ent. faecium. J. Clin. Microbiol. **38**, 3652-5, 2000.
10) Qamer S. et al: Use of colony morphology to distinguish different enterocccal strains and species in mixed culture from clinical specimens. J. Clin Microbiol **41**, 2644-6, 2003.
11) Ide L. et al: Arcanobact. pyogenes: spondylodiscitis in a Veterinary Surgeon: a plea for cooperation between medical and veterinary microbiologists in identification of causal agents of zoonotic infections. Clin. Microbiol. Newsletter **28**, 163-7, 2006.

© Copyright Rosco Diagnostica A/S

3.20 GLYCOSIDASES

General description
The chromogenic glycosidases tests are based upon enzymatic release of yellow-coloured nitrophenol from the substrates. Because the tests detect preformed enzymes non-growing suspensions can be used, and the tests are thus applicable also to microorganisms that do not grow in conventional test media. The tests are rapid and relatively inexpensive.

Range
The range of Glycosidase Diatabs comprises:

Beta-N-Acetylglucosaminidase	(NAG)	(50021)
Alpha-Fucosidase	(α-FUC)	(50111)
Beta-Fucosidae	(ß-FUC)	(59921)
Alpha-Galactosidase	(α-GAL)	(50211)
Beta-Galactosidase	(ONPG)	(50311)
Alpha-Glucosidase	(α-GLU)	(50411)
Beta-Glucosidase	(ß-GLU)	(50511)
Beta-Glucuronidase	(PGUA)	(50611)
Alpha-Mannosidase	(α-MAN)	(50711)
Beta-Xylosidase	(ß-XYL)	(50811)

Procedure
Prepare a dense "milky" bacterial suspension (at least McFarland No. 4) from the strain to be tested in 0.25 ml saline in a tube. Add one diagnostic tablet and close the tube. Incubate at 35-37 °C for **4 hours** or **overnight**.

Reading of the tests
Positive reaction: **Yellow**
Negative reaction: Colourless

When testing Beta-N-Acetylglucosaminidase only a strong yellow colour should be recorded as positive.

With strains that produce a yellow pigment (e.g. *Enterob. agglomerans*, Flavobacterium, Xanthomonas) or a red pigment (Serratia) use the bacterial suspension without the tablet (negative control) as control of colour, in order to facilitate the readings.

The tests are useful in identification of a wide variety of bacterial strains, including Enterobacteriaceae, non-fermenters, staphylococci, streptococci, anaerobes, neisseria, and haemophilus.

References General
1) Kilian M., Bülow P.: Rapid diagnosis of Enterobacteriaceae. Detection of bacterial Glycosidases. Acta Path. Microbiol. Scand. Sect. B., **84**, 245- 251, 1976.
2) Corbel M.J. et al: Identification of "Haemophilus somnus" by rapid tests for preformed enzymes. Letters in Appl. Microbiol. **3**, 13-15, 1986.
3) Haapasalo M., Ranta H., Shah H. et al: Biochemical and Structural Characterization of an Unusual Group of Gram-negative, Anaerobic Rods from Human Periapical Osteitis. J. Gen. Microbiol. **132**, 417-426, 1986.
4) Haapasalo M.: Bacteroides buccae and Related Taxa in Necrotic Root Canal Infections. J. Clin. Microbiol. **24**, 940-944, 1986.
5) Bruun B., Ursing J.: Phenotypic Characterization of Flavobacterium meningosepticum Strains Identified by DNA-DNA Hybridization. Acta Path. Microbiol. Scand. Sect. B, **95**, 41-47, 1987.
6) Murray P.R., Citron D.M.: General Processing of Specimens for Anaerobic Bacteria pp. 488-504 (500) in "Manual of Clinical Microbiology", 5th ed., ASM, 1991.
7) Kerr K.G., Rotowa N.A., Hawkey P.M., Lacey R.W.: Evaluation of the Rosco system for the identification of Listeria species. J. Med. Microbiol. **35**, 193-196, 1991.
8) Jousimies-Somer H.R. et al: Bacteroides, Porphyromonas, Prevotella, Fusobacterium and other anaerobic gram-negative bacteria. Manual Clin. Microbiology 6th Ed., ASM, 603-618, 1995.
9) Sumanen P., Barow E.J., Citron D.M., Strong C, Wexler H.M., Finegold S.M. Wodsworth Anaerobic Bacteriology Manual 5th Ed. Advanced Identification Methods (Level III) pages 65, 93, 152, 1993.

© Copyright Rosco Diagnostica A/S

10) Dumaz B. et al: Enzymatic profiles of Prevotella, Porphyromonas and Bacteroides species obtained with the APIZYM system and Rosco Diagnostic Tablets. Clin. Infect. Dis. **20** (suppl. 2) S192-S194, 1995.
11) Rautio M. et al: Characteristics of an unusual anaerobic pigmented gram negative rod isolated from normal and inflamed appendices. Clin. Infect. Dis. **25**, Suppl. 2, S107-S110, 1997.
12) Summanen P. et al: Wadsworth Anaerobic Bacteriology Manual. 5th ed. pages 49-50, 65, 93, 152, 157-9, 1993.

© Copyright Rosco Diagnostica A/S

3.20.1 BETA-N-ACETYLGLUCOSAMINIDASE (ß-NAG)

REF No. 50021

Results

1) Streptococci (*milleri*)

	NAG
S. intermedius	+
S. anginosus/constellatus	0

2) Actinomyces

Most strains are: Vanco S, Col R, Metro R^S, Cipro R.

	NAG	ONPG	PZA
A. europaeus	0	+	0
A. radingae	+	+	+
A. turicensis	0	0	0

3) Identification of *C. albicans* (4 h)

	NAG	PRO	42 °C	XYL	2h αGLU
Candida albicans	$+^0$	+	+	+	+
C. dublinensis	+	+	0	0	0
A) *Candida* spp.	0	+	.	.	.
B) *Candida* spp.	0	0	.	.	.

where A) comprises: *C. guilliermondii*, *C. lipolytica*, *C. lusitaniae*, *C. norvegensis*, *C. parapsilosis*, *Tor. Candida*.
where B) comprises: *C. glabrata*, *C. krusei*, *C. pseudotropicalis*, *C. rugosa* (NAG 0^+), *C. tropicalis* (NAG 0^+).

NAG = Beta-N-Acetylglucomaninidase D.T., ONPG = ONPG D.T., PZA = Pyrazinamidase D.T., PRO = Proline Amino-peptidase D.T., 42 °C = Growth at 42 °C in Sabouraud Glucose Agar, XYL = d-Xylose D.T.

4) Differentiation of most current *Candida* species (4 hours)

	Alk P	NAG	PRO	TRE	SUC	CYC	42 °C	2h αGLU
C. albicans	0	+	+	V	$+^0$	R (no zone)	+	+
C. glabrata	0	0	0 (V)	+	0	S	.	.
C. krusei	+	0	0	0	0	S	.	.
C. parapsilosis	0	0	+	0	$+^0$	R^S	.	.
C. tropicalis	+	0^+	0	V	$+^0$	R^S	.	.
C. dublinensis	.	$+^{'0}$	+	.	.	.	0	0

TRE = Trehalose D.T., SUC = Sucrose D.T., CYC = Cycloheximide D.T. (S ≥ 25 mm, R < 25 mm). AlkP = Alkaline Phosphatase D.T., 42 °C = Growth at 42 °C in Sabouraud Glucose Agar, 2h αGLU = Alpha-Glucosidase D.T. (incubation 2 hours).

5) Differentiation inside the *Clostridium clostridioforme* group (3,4)

	NAG	ONPG	RAF	IND
C. clostridioforme	0	+	+	0
C. bolteae	0	0	70	0
C. hathewayi	+	V	+	0
C. citroniae	0	0	0	+
C. aldenense	0	$+^0$	+	+

RAF = Raffinose.D.T, IND = Indole D.T.

© Copyright Rosco Diagnostica A/S

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Beta-N-Acetylglucosaminidase (p-Nitrophenyl-N-acetyl-β-D-glucosaminide)	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 25923

References (ß-NAG)

1) Jousimies-Somer H.R. et al: Anaerobic gram-negative bacilli and cocci. Manual of Clin. Microbiology 5th Ed. ASM, 538-552, 1991.
2) Niimi K. et al: Destinguishing Candida species by ß-N-acetylhexosaminidase activity. J. Clin. Microbiol. **39**, 2089-97, 2001.
3) Finegold S.M. et al: Clostridium clostridioforme: a mixture of 3 clinically imortant species. Eur. J. Clin. Microbiol. Infect. Dis. **24**, 319-24, 2005.
4) Warren Y.A. et al: Clostridium aldenense and Cl. citroniae sp nov isolated from human clinical infections. J. Clin. Microbiol. **44**, 2416-22, 2006.

© Copyright Rosco Diagnostica A/S

3.20.2 ALPHA-FUCOSIDASE (α-FUC)

REF No. 50111

Results

1) Streptococci

	α-FUC
S. gordonii	+
S. sanguinis	0

2) Anaerobe gram negative rods (Oxgall S, Brilliant Green S, Vanco 5 R, Kan R) non pigmented saccharolytic

	ARA	α-FUC	ß-GLU	ESC	ß-XYL	Remarks
Prevotella bergensis	+	0	0^{+}	+	+	IND 0, SUC 0, RAF 0, CEL +
Prevotella AIP-261-03	.	+	+	0	.	α-GAL +, SUC +
Prev. multisaccharivorax	.	0	+	+	.	
Prevotella disiens	0	0	0	0	0	α-GLU +
Prevotella oralis	0	+	+	+	0	
Prevotella bivia	0	+	0	0	0	Col R, CEL 0, RAF 0
Prevotella buccae	+	0	+	+	+	
Prevotella buccalis	0	+	0	+	0	
Prevotella multiformis	0	$+^{0}$	0	0	.	CEL +, RAF +
Prevotella baroniae	0	+	+	+	.	NAG +, α-GAL, ONPG +
Prevotella marshii	0	0	0	0	0	SUC 0, MAL +, GEL +, α-GLU
Prevotella massiliensis	0	0	0	0	0	IND +, OXI + KAN S non-saccharolyt

3) Porphyromonas human origin (Oxgall S, BrG S, Vanco 5 S)

	α-FUC	TRYP	IND	NAG	Remarks
P. asaccarolytica	+	0^{+}	$+^{0}$	0	
P. gingivalis	0	+	+	+	
P. endodentalis	0	0	+	0	
P. catoniae	+	+	0	+	Pigm 0
P.(levii like) = somerae	0	0	0	+	
Tannerella forsythensis	+	+	V	+	PGUA +, Vanco R
P. uenonis	0	.	+	0	

4) Anaerobe gram negative rods, pigmented (OXG S, BrG S, α–GLU+, TRYP 0)

	α-FUC	α-GAL	IND	CEL	LIP	NAG	Remarks
Prevotella melaninogenica	+	+	0	0	+	+	
P. intermedia/nigrescens	$+^{0}$	0	+	0	+	0	
Prevotella denticola	+	+	0	0	+	+	
Prevotella loescheii	+	+	0^{+}	+	+	+	
Prevotella corporis	0	0	0	0	0	0	TRYP $+^{0}$
Prevotella pallens	+	0	+	0	0	0	

© Copyright Rosco Diagnostica A/S

5) *Bacteroides fragilis* group (OXG R, BrG S, Vanco R, Kana 500 R, Col R, ESC $+^0$)

	α-FUC	PGUA	IND	ß-GLU	α-GAL	TRE	RHAM	ARA	CAT	Remarks
Bacteroides fragilis	+	.	0	+	+	0	0	0	+	
Bacteroides vulgatus	+	.	0	0	+	0	+	+	0^+	ESC 0, LAP 0
Parabacteroides distasonis	0	0	0	+	+	+	+	0	$+^0$	
Bacteroides cacae	95	.	0	+	+	+	$+^0$		0^+	
Parabacteroides merdae	0	+	0	0	+	+	+	0^+	0^+	PYR +
Parabacteroides finegoldii	0	0	(0)	0	+	0	.	+	.	ADH 0, LAP 0
Parabacteroides goldsteinii	0	+	0	+	+	+	.	0	V	ß-XYL +
Bacteroides plebeius	+	+	0	+	+	0	+	+	0	NAG +, ESC +, CEL +
Bacteroides coprocola	+	0	0	+	+	0	+	0	0	LAP 0
Bacteroides massiliensis	+	0	0	+	+	0	0	0	0	LAP +
Bacteroides dorei	+	+	0	+	+	0	+	+	0	NAG + ESC 0, CEL 0
Bact. thetaiotaomicron	+	.	+	+	+	$+^{wk}$	+	+	+	SAL 0^+, LAP 0
Bacteroides ovatus	+	.	+	+	+	+	+	+	$+^0$	SAL +
Bacteroides uniformis	+	.	+	+	+		0	+	0^+	SAL +
Bacteroides stercoris	$+^0$	.	+	+	0	0	+	0	0	ADH +, CEL 0
Bacteroides eggerthii	0	.	+	+	0	0	$+^0$	+	0	SUC 0
Bacteroides nordii	0	.	+	+	+	0	+	0	0	ADH 0, CEL +
Bacteroides salyersae	0	.	+	+	+	0	+	+	0	SUC +
Bacteroides intestinalis	+	0	+	+	+	0	+	+	.	SAL 0

α-FUC = Alpha-Fucosidase D.T., TRYP = Trypsin D.T., IND = Indole D.T., β-GLU = Beta-Glucosidase D.T., ESC = Esculin Hydrolysis D.T., α-GAL = Alpha-Galactosidase D.T., CEL = Cellobiose D.T., OXG = Oxgall D.T., BrG = Brilliant Green D.T., LIP = Lipase, TRE = Trehalose D.T., ARA = Arabinose D.T., CAT = Catalase, ß-XYL = Beta-Xylosidase D.T., NAG = Beta-N-Acetylglucosaminidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., PGUA = Beta-Glucuronidase D.T., SAL = Salicin D.T., COL = Colistin 10 µg D.T. (S ≥ 13 mm, R ≤ 10 mm), α-GLU = Alpha-Glucosidase D.T., Oxgall D.T. (S ≥ 10 mm, R < 10 mm), BrG = Brilliant green D.T. (S ≥ 10 mm, R < 10 mm), Kana 500 = Kanamycin 500 Neo-S (S ≥ 10 mm, R < 10 mm) Col = Colistin 10 µg Neo-S (S ≥ 10 mm, R < 10 mm) Vanco = Vancomycin 5 µg Neo-S (S ≥ 20 mm, R ≤ 18 mm), ADH = Arginine Dihydrolase D.T., SUC = Sucrose D.T. , RAF = Raffinose D.T., OXI = Oxidase D.T., α-GAL = Alpha-Galactosidase D.T., MAN = Mannitol D.T., ONPG = ONPG.D.T., MAL = Maltose D.T., GEL = gelatinase, ADH = Arginine Dihydrolase. D.T., LAP = Leucine Aminopeptidase D.T., RHAM = l-Rhamnose D.T.

6) <u>Pigmented</u> gram negative rods (anaerobes)

	IND	α-GLU	ONPG	α-FUC	NAG	LIP	Remarks
Prev. denticola/ melaninogenica/loescheii	0	+	+	+	+	+	Vanco R
Prev. intermedia/nigrescens	+	+	0	+	0	+	Vanco R
Prev. pallens	+	+	0	+	0	0	Vanco R
Prev. corporis	0	+	0	0	0	0	Vanco R
Prev. bivia	0	+	+	+	+	0	Vanco R, RAF 0
Prev. disiens	0	+	0	0	0	0	Vanco R
Prev. multiformis	0	+	+	$+^0$	.	0	Vano R, RAF +
Porph. asaccharolytica	+	0	0	+	0	0	TRYP 0, Vanco S
Porph. gingivalis	+	0	+	0	+	0	TRYP +, Vanco S
Porph. endodentalis	+	0	0	0	0	0	TRYP 0, Vanco S
Prev. massiliensis (non-pigm.)	+	0	0	0	.	.	Vanco R, Kana S, OXI +

IND = Indole D.T., α-GLU = Alpha-Glucosidase D.T. α-FUC = Alpha-Fucosidase D.T., NAG = Beta-N-Acetylglucosaminidase D.T., LIP = Lipase, TRYP = Trypsin D.T., Vanco = Vancomycin 5 µg Neo-S (S ≥ 20 mm, R ≤ 18 mm), RAF = Raffinose D.T.

© Copyright Rosco Diagnostica A/S

7) Differentiation of Bacteroides/Parabacteroides (5)

	α-FUC	IND
Bacteroides (IND 0)	+	0
Bacteroides (IND +)	V	+
Parabacteroides	0	0

α-FUC = Alpha-Fucosidase D.T., IND = Indole D.T.

8) Differentiation of Parabacteroides (5,6) (OXG R, Vanco R, Kan 500 R, Col R)

	α-FUC	IND	ß-GLU	PGUA	PYR	CAT	Remarks
P. distasonis	0	0	+	0	0	+	
P. merdae	0	0	0	+	+	0	
P. goldsteinii	0	0	+	+	0	V	ß-XYL +
P. johnsonii	0	0	0	+	0	+	

α-FUC = Alpha-Fucosidase D.T., IND = Indole D.T., ß-GLU = Beta-Glucosidase D.T, PGUA = Beta-Glucuronidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., CAT = catalase.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Alpha-Fucosidase (p-Nitrophenyl-α-L-Fucosidase)	*B. fragilis* ATCC 25285	*E. coli* ATCC 25922

References (α-FUC)

1) Heltberg et al: The cultivation and rapid enzyme identification of DF-2. Eur. J. Clin. Microbiol. **3**, 241-3, 1984.
2) Jousimies-Somer H.R. et al: Anaerobe gram-negative bacilli and cocci, Manual of Clin.Microbiology 5th Ed. ASM, 538-552, 1991, 6th Ed. ASM, 603-620, 1995, and 8th Ed. ASM 888-896, 2003.
3) Könönen E. et al: Biohemical and genetic characterization of a Prevotella intermedia/nigrescens-like organism. Intl. J. Syst. Bacteriol. **48**, 39-46, 1998.
4) Könönen E. et al: Phylogenetic characterization and proposal of a new pigmented species to the genus Prevotella: Prevotella pallens sp. nov. Intl. J. Syst. Bacteriol. **48**, 47-51, 1998.
5) Sakamoto M. et al: Reclassification of B. distasonis, B. goldsteinii and B. merdae as Parabacteroides distasonis gen. nov. comb. nov., Parabacteroides goldsteinii comb. nov. and Parabact. merdae comb. nov. Intl. J. System Evol. Microbiol. **56**, 1599-1605, 2006.
6) Sakamoto M. et al: Parabacteroides johnsonii sp. nov., isolated from human faeces. Intl. J. System Evol. Microbiol. **57**, 293-296, 2007.

© Copyright Rosco Diagnostica A/S

3.20.3 BETA-FUCOSIDASE (ß-FUC)

REF No. 59921

Results

1) Streptococcus "milleri" anginosus group (ADH +, CAT 0, ESC +, VP +, MAN 0, PYR 0, SOR 0)

	ß-FUC	NAG	ß-GLU	RAF	α-GLU
S. anginosus	0	0	+	V	0^{+}
S. constellatus	0	0	0	0	$+^{0}$
S. constellatus subsp. *pharyngis*	+	+	$+^{0}$	0	.
S. intermedius	+	+	V	0^{+}	+

NAG = Beta-N-Acetylglucosaminidase D.T., ß-GLU = Beta-Glucosidase, RAF = Raffinose D.T., α-GLU = Alpha-Glucosidase D.T.

2) Differentiation of Group C and G beta-haemolytic streptococci

	VP	PGUA	ß-GLU	ß-FUC	SORB	TRE
S. anginosus	+	0	+	0	0	$+^{0}$
S. constellatus	+	0	0	0	0	.
S. dysgalactiae * subsp. *equisimilis*	0	+	V	0	0	+
S. constellatus subsp. *pharyngis*	+	0	$+^{0}$	+	0	.
S. equi subsp. *zooepidermicus*	0	+	V	0	+	0

* may be alpha-haemylytic sometimes.

VP = Voges Proskauer D.T., PGUA = Beta-Glucuronidase D.T., ß-GLU = Beta-Glucosidase D.T., ß-FUC Beta-Fucosidase D.T., SORB = Sorbitol D.T., TRE = Trehalose D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Beta-Fucosidase (p-Nitrophenyl-ß-D-fucopyranoside)	*S. intermedius* ATCC 27335	*E. coli* ATCC 25922

References

1) Whiley R.A. et al: Phenotypic differentiation of S. intermedius S. constellatus and S. anginosus strains within the "S. milleri group". J. Clin. Microbiol. **28**, 1497-1501, 1990.
2) Whiley R.A. et al: A study of small-colony, beta-haemolytic, Lancefield group C streptococci within the anginosus group: description of S. constellatus subsp. pharyngis subsp. nov., associated with the human throat and pharyngitis. I.J.S.E.M. **49**, 1443-9, 1999.
3) Gray T.: Streptococcus anginosus group: clinical significance of an important group of pathogens. Clin. Microbiol. Newslettter **27**, 155-9, 2005.

© Copyright Rosco Diagnostica A/S

3.20.4 ALPHA-GALACTOSIDASE (α-GAL)

REF No. 50211

Results

1) Nutritionally variant streptococci (NVS) = *Abiotropia* and *Granulicatella* spp. (PYR +, LAP +)

	α-GAL	PGUA	NAG	ADH
A. defectiva	+	0	0	0
G. adiacens	0	+	0	0
G. elegans	0	0	0	+
G. balaenopterae	0	0	+	+

α-GAL = Alpha-Galactosidase D.T., PGUA = Beta-Glucuronidase D.T., NAG = Beta-N-acetylglucosaminidase D.T. and ADH = Arginine Dihydrolase D.T., LAP = Leucine Aminopeptidase D.T.

2) Anaerobe gram negative rods <u>pigmented</u> (Oxgall S, Brilliant Green S, α-GLU +, TRYP 0)

	α-GAL	α-FUC	IND	CEL	LIP
Prevotella melaninogenica	+	+	0	0	+
P. intermedia/nigrescens	0	+	+	0	+
Prevotella denticola	+	+	0	0	+
Prevotella loescheii	+	+	0	+	+
Prevotella corporis	0	0	0	0	0
Prevotella pallens	0	+	+	0	0

α-GAL = Alpha-Galactosidase D.T., α-FUC = Alpha-Fucosidase D.T., IND = Indole D.T. and CEL = Cellobiose D.T., LIP = Lipase Oxgall D.T. (S ≥ 10 mm, R < 10 mm), Brilliant green D.T. (S ≥ 10 mm, R < 10 mm), α-GLU = Alpha-Glucosidase D.T., TRYP = Trypsin D.T.

3) Differentiation of <u>Aerotolerant</u> *Clostridium* spp. (Vanco S, Kana S, Col R)

	α-GAL	ONPG	PYR	Gel	SORB	HEM
Clostridium tertium	+	+	0	0	0	α/ ß
Clostridium histolyticum	+	0^{+}	+	+	0	0
Clostridium intestinale	.	.	.	0	+	beta

α-GAL = Alpha-Galactosidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., Gel = Gelatine hydrolysis, SORB = Sorbitol, HEM = hemolysis.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Alpha-Galactosidase (p-Nitrophenyl- α-D-Galactopyranoside 0.3)	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853

References (α-GAL)

1) Ruoff K.L.: Nutritionally variant streptococci. Clin. Microbiol. Reviews **4**, 184-90, 1991.
2) Steyaert S. et al: Septicemia in neutropenic patients infected with Clostridium tertium resistant to Cefepime and other expanded-spectrum cephalosporins, J. Clin. Microbiol. **37**, 3778-9, 1999.
3) Christensen J.J., Facklam R.R.: Granulicatella and Abiotrophia species from human clinical specimens. J. Clin. Microbiol. **39**, 3520-3, 2001.

© Copyright Rosco Diagnostica A/S

3.20.5 ONPG - Beta-Galactosidase (ONPG)

REF No. 50311

Results

1) Actinobacillus/Pasteurella

	ONPG	URE	IND
Actinobacillus spp.	+	+	0
Pasteurella spp.	0	0^+	+

2) Actinomyces

Most strains are: Vanco S, Col R, Metro R^S, Cipro R.

	ONPG	NAG	PZA
A.. europaeus	+	0	0
A. radingae	+	+	+
A. turicensis	0	0	0

ONPG = ONPG D.T., URE = Urease D.T., IND = Indole D.T., NAG = Beta-N-Acetylglucosaminidase D.T. and PZA = Pyrazinamidase D.T.

3) HACEK group and miscellaneous gram negative rods/cocobacilli (Capnocytophaga, *Pasteurella* spp.)

	γ-GLU	OXI	CAT	SUC	α-GLU	ONPG	TRYP	IND	NIT	Remarks
*Aggregatibacter aphrophilus**	+	V	0	+	$+^0$	+	0	0	+	ALA +
A. actinomycetemcomitans	+	0^+wk	+	0	0	0	0	0	+	LAP +
Cardiob. hominis	+	+	0	+	0	0	+	+ wk	0	MAN +, TTR +
Cardiob. valvarum	·	+	0	V	+	0	+	$+^0$	0	MAN 0, TTR +
Eikenella corrodens	0	+	0	+	0	+	0	0	+	LDC $+^0$, ODC +, PRO +
Kingella spp.	0	+	0	V	0	0	·	V	0^+	Col R
Capnocytophaga spp.	·	0	0	+	+	$+^0$	$+^0$	0	$+^0$	
Capn. canimorsus	·	+ wk	+	0	+	+	+	0	0	α-FUC +, ADH +
Capn. cynodegmi	·	+	+	+	+	+	+	0	+	
Past. multocida	0	+	+	+	V	0	0	$+^0$	+	ODC $+^0$, PRO 0
Mannh. haemolytica	·	+	$+^0$	+	·	$+^0$	0	0	+	ODC 0, α-FUC +

γ-GLU = Gamma-Glutamyl Aminopeptidase D.T., OXI = oxidase, CAT = catalase, SUC = Sucrose D.T., α-GLU = Alpha-Glucosidase D.T., TRYP = Trypsin D.T., IND = Indole D.T., NIT = Nitrate reduction, ALA = Porphyrin D.T., Col = Colistin 10 µg D.T.(S ≥ 13 mm, R ≤ 10 mm), α-FUC = Alpha-Fucosidase D.T.

* *Aggregatibacter aphrophilus* cover the previous *H. aphrophilus* and *H. paraphrophilus*.

4) Differentiation of *Actinobacillus* spp.

Most strains are: URE +, ONPG +, NO_3, +, ADH 0, ODC 0, IND 0, O/129 S.

	OXI	CAT	αGAL	αGLU	ßXYL	ßGLU	SOR	TRE	MAN	Remarks
Actinobacillus hominis	+	0	+			V	0	+	+	
A. equuli ssp. *equuli*	V	+wk	+	+	+	0	0	+	+	
A. equuli ssp. *haemolyticus* (B-11)	+	+wk	+	V	$+^0$	$+^0$	V	+	0^+	ß-haem $+^0$
A. lignieresii	$+^0$	$+^0$	0	0	0	0	0	0	+	LACT +
A. pleuropneumoniae	0^+	V	0			V	0	0	+	LACT 0
A. suis	$+^0$	+	+			+	0	+	0	
A. capsulatus	+	+	+			$+^0$	+	+	+	
A. Bisgaard taxon 8	+	+	+			0	0	0	+	

© Copyright Rosco Diagnostica A/S

	OXI	CAT	αGAL	αGLU	ßXYL	ßGLU	SOR	TRE	MAN	Remarks
A. arthritidis (B-9)	+	+	+	0	0	0	+	0	+	
A. genomospecies 2	+	+	+	0	V	0	0	0	+	
A. ureae	0^{+}	V	0			0	0^{+}	0	+	ONPG 0, URE $+^{R}$
"Pasteurella pneumotropica"	+	+	V			0	0	V	0	IND +, ODC +

OXI = Oxidase D.T., CAT = Catalase, αGAL = Alpha-Galctosidase D.T., αGLU = Alpha-glucosidase D.T., ßXYL = Beta-Xylosidase D.T., ßGLU = Beta-Glucosidase D.T., SOR = Sorbitol D.T., TRE = Trehalose D.T., MAN = Mannitol D.T., ß-haem = beta haemolysis, LACT = Lactose D.T., URE = Urease D.T., $+^{R}$ = rapidly positive, IND = Indole D.T., ODC = Ornithine Decarboxylase D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
ONPG (Beta-Galactosidase) (o-Nitrophenyl-β-D-Galactopyranoside)	*E. coli* ATCC 25922	*S. aureus* ATCC 25923

References (ONPG)

1) Bruun B., Ursing J. Phenotypic charaterization of Flavobacterium meningosepticum strains identified by DNA-DNA hybridization. Acta path. microbiol. immunol. scand. Section B, **95**, 41-47, 1987.
2) Ashhurst-Smith C. et al: Actinobacillus equuli septicemia: an unsual zoonotic infection. J. Clin. Microbiol. **36**, 2789-90, 1998.
3) Friis Møller A. et al: Clinical significance and taxonomy of Actinobacillus hominis. J.Clin. Microbiol. **39**, 930-5, 2001.
4) Christensen H. et al: Final classification of Bisgaard taxon 9 as A. Actinobacillus arthritidis sp. nov. and recognition of a novel genomospecies for equine strains of A. lignieresii. IJSEM **52**, 1239-46, 2002.
5) Christensen H. et al: Raclassification of equine isolates previously reported as A. equuli, variants of A. equuli, A. suis or Bisgaards taxon 11 and proposal of A. equuli ssp. equuli ssp. nov. and A. equuli ssp. haemolyticus ssp. nov. IJSEM **52**, 1569-76, 2002.

© Copyright Rosco Diagnostica A/S

3.20.6 ALPHA GLUCOSIDASE (α-GLU) BETA-GLUCOSIDASE (ß-GLU)

REF No. 50411
REF No. 50511

Results

1) ***Gardnerella vaginalis***
CAT 0, OXI 0

	α-GLU	ß-GLU	SPS	HIP	Remarks
Gardnerella vaginalis	+		S (≥10 mm)	+	PRO +
Lactobacillus vaginalis	+	.	R	V	
Corynebacteria vaginalis	V	.	R	V	
Bifidobacterium	+	.	R	0	
Atopobium vaginae	0	0	R	.	PRO +, ADH +, LAP +, Metro R, Vanco S.

2) ***Enterobacter sakazakii*** **(2,3)**

	α-GLU
Enterobacter sakazakii	+
Enterobacte cloacae	0
Enterobacter aerogenes	0
Enterobacter agglomerans	0

α-GLU = Alpha-Glucosidase D.T., β-GLU = Beta-Glucosidase D.T., SPS = S.P.S. D.T., HIP = Hippurate Hydrolysis D.T.

3) Differentiation of *C. albicans* from *C. dublinensis*

	α-GLU (2h)
Candida albicans	+
Candida dublinensis	0

α-GLU (2h) = Alpha-Glucosidase D.T. (incubation 2 hours).

4) Phenotypic patterns of Aerococcus urinae (4)

	ß-GLU	PGUA	ESC
A. urinae phenotype I	0	+	0
A. urinae phenotype II	+	0	+

ß-GLU = Beta-Glucosidase D.T., PGUA = Beta-Glucosidase D.T., ESC = Esculin Hydrolysis D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Alpha-Glucosidase (p-Nitrophenyl-α-D-Glucopyranoside)	*S. maltophilia* ATCC 13637	*P. aeruginosa* ATCC 27853
Beta-Glucosidase (p-Nitrophenyl-ß-D-Glucopyranoside)	*K. pneumoniae* ATCC 13883	*Morganella morganii* ATCC 25830

© Copyright Rosco Diagnostica A/S

References (α-GLU, ß-GLU)

1) Bastida Vilá M.T. et al: Gardnerella vaginalis bacteremia in an adult male. J. Clin. Microbiol. Infect. Dis. **16**, 400-1, 1997.
2) Muytiens H.L. et al: Enzymatic profiles of Enterobacter sakazakii and related species with special reference to α-glucosidase reaction and reproducibility of the test system. J. Clin. Microbiol. **20**, 684-6, 1984.
3) Poterac. E. sakazakii unexpectedly widespread in some food-processing plants. ASM news, **70**, 109, 2004.
4) Christensen J.J. et al: Aerococcus urinae: polyphasic characterisation of the species. APMIS **113**, 517-525, 2005.

© Copyright Rosco Diagnostica A/S

3.20.7 BETA-GLUCURONIDASE (PGUA)

REF. No. 50611

Beta-Glucuronidase (PGUA) Diatabs are useful in the presumptive identification of ***Escherichia coli.*** As *E. coli* is the ethiological agent of approx. 80 % of urinary tract infections, a simple, specific, rapid and accurate method for its identification is very useful.

Procedure
Prepare a dense bacterial suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one PGUA tablet and close the tube. Incubate at 35-37 °C for **4 hours** (or **overnight**).

Reading of the test
Positive reaction: **Yellow**
Negative reaction: Colourless

Approx. 94 % of *E. coli* are positive for the PGUA test. Among the other Enterobacteriaceae only some Shigella and Salmonella (approx. 30 %) are found positive. Strains of *Citrob. freundii* and *Enterobacter cloacae* have been found positive in uncommon cases.

Results

1) Enterobacteriaceae

	PGUA
E. coli	94
Salmonella spp.	V
Shigella spp.	V
Other	0

2) *Arcanobacterium haemolyticum* biotypes

	PGUA	SUC	Infection
A. haemolyticum smooth	0	41	wounds
A. haemolyticum rough	97	0	respiratory

PGUA = Beta-Glucuronidase D.T., SUC = Sucrose D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Beta-Glucuronidase (PGUA) (p-Nitrophenyl- ß -D glucuronic acid)	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 13883

References (PGUA)
1) Dibb W.L., Bottolfsen K.L.: Evaluation of Rosco diagnostic beta glucuronidase tablets in the identification of urinary isolates of Escherichia coli. Acta Path. Microbiol. Scand. Sect.B. **92**, 261 264, 1984.
2) Hansen W., Yourassowsky E.: Detection of beta-glucuronidase in lactose-fermenting members of the family Enterobacteriaceae and its presence in bacterial urine cultures. J. Clin. Microbiol. **20**, 1177, 1179, 1984.
3) Pérez J.L., Berrocal C.I., Berrocal L.: Evaluation of a commercial beta-glucuronidase test for the rapid identification of Escherichia coli. J. Applied Bacteriol. **61**, 541-545, 1986.
4) Casals J.B., Pringler N.: Rapid Identification of E. coli with a Double Test Tablet: Beta Glucuronidase (PGUA)/Indole". 4th European Congress of Clinical Microbiology, Nice, 1989, poster 514.
5) Domínguez A., Alcaide F., Pulido A., Ayats J., Pérez J.L., Martín R.: Use of a Commercial Double-Test Tablet (Rosco PGUA/Indole) for Screening of Escherichia coli. Diagn. Microbiol. Infect. Dis. **15**, 291-294, 1992.
6) Vandepilte J. et al: Basic laboratory producedures in Clinical Bacteriology WHO Geneva, page 35-36 and 109, 1991.
7) Carlson P. et al: Biotypes of Arcanobacterium haemolyticum. J. Clin. Microbiol. **32**, 1654-7, 1994.

© Copyright Rosco Diagnostica A/S

3.20.8 ALPHA-MANNOSIDASE (α-MAN)

REF No. 50711

Results

1) Actinomyces

	α-MAN
Actinomyces gerencseriae	+
Actinomyces israelii	0

2) Arcanobacterium / Listeria / Corynebacterium

	ß-FUC	α-MAN	PRO	PYR	VP(24h)	TRIB	XYL	CAT	FOSFO	Remarks
A. pyogenes	+	0	+	82	+	0	+	0	S	
A. haemolyticum	+	+	+	0	0	70	0	0	S	
Dermabacter hominis	.	+	.	+	.	+	V	+	S	LDC +
Listeria spp.	.	$+^0$	0	0	+	.	V	+	R	
Corynebacterium spp.	0	0	+	V	.	.	.	.	R	
Erysipelothrix spp.	+	0	0	.	.	.	.	.	.	Vanco R

ß-FUC = Beta-Fucosidase D.T., α-MAN = Alpha-Mannosidase D.T., PRO = Proline Aminopeptidase. D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., VP (24h) = Voges Proskauer D.T. (24 hours' incubation), TRIB = Tributyrin D.T., XYL = Xylose D.T., CAT = Catalase, FOSFO = Fosfomycin Neo-S (S ≥ 30 mm, R ≤ 28 mm).

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Alpha-Mannosidase (p-Nitrophenyl-α-Mannopyranoside)	*L. monocytogenes* ATCC 19115	*E. coli* ATCC 25922

References (α-MAN)

1) Carlson P. et. al: Alpha mannosidase: a rapid test for identification of Arcanobacterium haemolyticum. J. Clin. Microbiol. **32**, 854-5, 1994.
2) von Gravenitz A: Alpha mannosidase in Arcanobacterium haemolyticum. J. Clin. Microbiol. **32**, 2883, 1994.

© Copyright Rosco Diagnostica A/S

3.20.9 BETA-XYLOSIDASE (ß-XYL)

REF No. 50811

Results

1) Acinetobacter

	ß-XYL	γGLU
Acinetobacter baumaniii /calcoaceticus	$+^0$	$+^0$
A. lwoffi	0	0

2) Propionibacteria, Metro I/R

	ß-XYL	ONPG	Remarks
Propionibacterum acnes	0	+	(CAT +, IND +)
Propionibacterium. avidum	+	+	
P. granulosum	0	0	
P. propionicum	0	$+^0$	(CAT 0, IND 0)

3a) Enterobacteriaceae

	ß-XYL	PYR
Klebsiella spp.	+	+
Enterobacter spp.	+	+
Yersinia spp.	V	$+^0$
Citrobacter spp.	0	+
Serratia spp.	0	+
Citrobacter amalonaticus	V	+
Serratia rubidaea	V	+
Other enterobacteriaceae	0	0

3b) Klebsiella/Enterobacter/Serratia

	PYR	ß-XYL	ODC
Klebsiella spp.	+	+	0
Enterobacter spp.	+	+	+
Serratia spp.	+	0	+

4) Capnocytophaga (PYR +, TRYP +, OXI 0, CAT 0)

	ß-XYL	NAG
Capnocytophaga gingivalis	0	0
Capnocytophaga sputigena	+	$+^0$
Capnocytophaga ochracea	0	+

β-XYL = Beta-Xylosidase D.T., ONPG = ONPG D.T., CAT = catalase, IND = Indole D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., NAG = Beta-N-Acetylglucosaminidase D.T., γ-GLU = Gamma-Glutamyl Aminopeptidase D.T. OCD = Ornithine Decarboxylase D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Beta-Xylosidase (p-Nitrophenyl ß-D-xylopyranoside)	*K. pneumoniae* ATCC 13883	*E. coli* ATCC 25922

© Copyright Rosco Diagnostica A/S

References (ß-XYL)

1) Jousimies-Somer H. et.al: Bacteroides, Porphyromonas, Prevotella, Fusobacterium and other anaerobic gram-negative bacteria. Manual Clinical Microbiology 6th Ed., 603-618, 1995.
2) Murray P.R., Citron D.M.: General processing of specimens for anaerobic bacteria. Manual Clinical Microbiology 5th Ed., 488-500, 1991.

© Copyright Rosco Diagnostica A/S

3.21 HIPPURATE HYDROLYSIS (HIP)

REF No. 56711

Diagnostic Tablets for determinating the ability of bacterial strains to hydrolyze hippurate by the action of the enzyme hippurate hydrolase. The tablets contain sodium hippurate which is split into benzoic acid and the amino acid glycine. The latter is detected in the test by addition of Ninhydrin solution.

$$\text{Hippuric acid} + H_2O \xrightarrow{\text{Hippurate hydrolase}} \text{Benzoic acid} + \text{Glycine}$$

Procedure
Prepare a dense suspension of the strain (at least McFarland No. 4) to be tested in 0.25 ml saline in a tube. Add one Hippurate Hydrolysis Diagnostic Tablet, close the tube and incubate for **4 hours** or **overnight** at 35-37 °C.

For Campylobacter it is important to harvest bacteria from blood-agar (TSA or Columbia agar + blood) and to use a high inoculum.

After incubation add 5 drops of **Ninhydrin 3.5% sol.** (91731), close the tube and **reincubate** for **10 minutes** at 35-37 °C. Read within 5 minutes.

Reading of the test
Positive reaction: **Deep purple - blue**
Negative reaction: Colourless, light yellow or occasionally a faint tinge of purple

Do not reincubate longer than 10 minutes as false positives may result. Do not use reagents other than ninhydrin to make the colour reaction. The test is useful in the presumptive identification of Group B streptococci, *Gardnerella vaginalis*, and *Campylobacter jejuni*.

Results

1a) *Streptococcus* spp.

	HIP
Group B streptococci	+
Other beta-haemolytic Streptococci (except group D)	0

1b) Streptococci from subclinical mastitis

	HIP	ESC	PGUA	PYR	CAMP
S. agalactiae (B)	+	0	V	0	+
S. dysgalactiae subsp. *dysgalactiae* (C)	0	0^+	+	0	0
S. uberis (E, P, U, G)	$+^0$	+	+	+	0^+
S. parauberis	$+^0$	+	0	+	0
S. canis (G)	0	$+^0$	0^+	V	+

ESC = Esculin Hydrolysis D.T., PGUA = Beta-Glucuronidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., CAMP = CAMP reaction.

2a) *Campylobacter* spp.

	HIP
Campylobacter jejuni	+
Helicobacter westmeadii	+
Other campylobacter / Helicobacter	0

© Copyright Rosco Diagnostica A/S

2b) Differentiation of enteropathogenic Campylobacters / *Arcobacter butzleri*

	HIP	IAC	CAT	CLOTN	25 °C	COL
C. coli	0	+	+	R	0	R
C. jejuni	+	+	$+^{0}$	R^{S}	0	R
C. lari	0	0	+	R	0	R
C. upsaliensis	0	+	0 wk	S	0	R
C. fetus	0	0	+	S	+	R
Arcobacter butzleri	0	$+^{0}$	V	R	+	S

IAC = Indoxyl Acetate D.T., CAT = catalase, CLOTN = Cephalothin Neo-S (S ≥ 16 mm, R < 16 mm), 25 °C = Growth at 25 °C, COL = Colistin.

3) *Gardnerella vaginalis*
CAT 0, OXI 0

	HIP	SPS	
Gardnerella vaginalis	+	S	(≥10 mm), PRO +
Bifidobacteria	0	R	
Lactobacilli/dipftheroids	V	R	
Atopobium vaginae	.	R	PRO +, ADH +, LAP +, Metro R, Vanco S

4) Nutritionally variant streptococci (NVS) = *Abiotropia* spp. and *Granulicatella* spp. (PYR +, LAP +)

	HIP	PGUA	α-GAL	ADH
A. defectiva	0	0	$+^{0}$	0
G. adiacens	0	$+^{0}$	0	0
G. elegans	+	0	0	+
G. baldenopterae	0	0	0	+

5) Differentiation of *Facklamia* spp.

	HIP	ADH	SUC	TRE
Facklamia hominis		+	V	0
Facklamia ignava	+	V	+	0^{+}
Facklamia languida	0	0	0	+
Facklamia sourekii	+	0	+	+
Facklamia tabacinasalis	0	0	+	+

HIP = Hippurate Hydrolysis D.T., PGUA = Beta-Glucuronidase D.T., α-GAL = Alpha-Galactosidase D.T., ADH = Arginine Dihydrolase D.T., SUC = Sucrose D.T., TRE = Trehalose D.T.

6) Catalase negative cocci from milk (8)

	LAP	PYR	HIP	INU	RAF
S. uberis	+	+	+	$+^{0}$	0
S. bovis	+	0	0	V	+
S. dysgalactiae	+	0	0	0	0
Aerococcus spp.	0	+	-	-	-
Enterococcus/lactococcus	+	+	V	0	V

LAP = Leucine Aminopeptidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., HIP = Hippurate Hydrolysis D.T., INU = Inulin D.T., RAF = Raffinose D.T.

7) Differentiation of *Mobiluncus* spp.

	HIP	ADH	ONPG	α-FUC	α-GLU
Mobiluncus curtisii	+	+	$+^{0}$	0	+
Mobiluncus mulieris	0	0	0	V	+

ADH = Arginine Dihydrolase D.T., α-FUC = Alpha-Fucosidase D.T., α-GLU = Alpha-Glucosidase D.T.

© Copyright Rosco Diagnostica A/S

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Hippurate Hydrolysis (Hippuric acid Sodium-salt)	*S. agalactiae* ATCC 12386	*S. pyogenes* ATCC 12344

References

1) Bastida Vilá M.T. et al: Gardnerella vaginalis bacteremia in an adult male. Eur. J. Microbiol. Infect. Dis. **16**, 400-1, 1997.
2) Roggenkamp A. et al: Abiotropia elegans sp. nov. a possible pathogen in patients with culture negative endocarditis. J. Clin. Microbiol. **36**, 100-4, 1998.
3) Sorlin P. et al: Recurrent "Flexispira rappini" bacteremia in an adult patient undergoing hemodyalisis: case report. J. Clin. Microbiol. **37**, 1319-23, 1999.
4) Sato S. et al: Abiotropia elegans strains comprise 8% of the nutririonally variant streptococci isolated from the human mouth. J. Clin. Microbiol. **37**, 2553-6, 1999.
5) Lawson P.A. et al: Facklamia languida sp. nov. isolated from human clinical specimens. J. Clin. Microbiol. **37**, 1161-4, 1999.
6) Christensen J.J. Facklam R.R.: Granulicatella and Abiotrophia species from human clinical specimens. J. Clin. Microbiol. **39**, 3520-3, 2001.
7) On S.L.W.: Identification methods for Campylobacters, Helicobacters and related organisms. Clin. Microbiol. Reviews **9**, 405-22, 1996.
8) Fortin M et al: Identification of catalase negative, non-beta-haemolytic gram positive cocci isolates from milk samples. J. Clin. Microbiol. **41**, 106-109, 2003.
9) Hoyles L et al: Transfer of members of the genus Falcivibrio to the genus Mobiluncus and amended description of the genus Mobiluncus. System. Appl. Microbiol. **27**, 72-83, 2004.

© Copyright Rosco Diagnostica A/S

3.22 INDOXYL ACETATE (IAC)

REF No. 59551

Diagnostic tablets that are useful in the identification of *Campylobacter* spp. *C. jejuni*, *C. coli* and *C. upsaliensis* are positive while other *Campylobacter* spp. are negative. The related species *Arcobacter cryaerophilus*, *Arcobacter butzleri,* and *Helicobacter fennelliae* and occasionally Helicobacter cinaedi (weak pos.) also give a positive reaction while *Helicobacter pylori* is negative. Indoxyl Acetate is packed in vials of 15 tablets. **Store at 2-8°C.**

Principle
The diagnostic tablets are used to detect the presence of acetate esterase in microorganisms. Organisms possessing acetate esterase activity hydrolyze indoxyl acetate into acetic acid and free indoxyl. Free indoxyl reacts with oxygen, which results in a blue/green colour (positive).

Procedure
Prepare a dense "milky" suspension equivalent to at least McFarland No.4 from freshly-grown colonies into 0.25 ml saline in a small tube. Add one Indoxyl Acetate Diagnostic Tablet and close the tube. Incubate at 37 °C in ambient air for **4 hours** or **18-24 hours**.

Reading of the test
Positive reaction: **Blue, green sediment**
Negative reaction: Colourless, slightly coloured supernatant (sediment not blue)

Results

1) Campylobacter/Helicobacter

Most strains are: OXI +, CAT +.

	IAC	γGLU
Campylobacter jejuni	+	V
Campylobacter coli	+	0
Campylobacter upsaliensis	+	·
Helicobacter fennelliae	+	0
H. salomonis/bizzozeroni	+	+
Campylobacter lari	0	·
Helicobacter pylori	0	+
H. felis/cynogastricus	0	+

2) *Helicobacter* spp. isolated from <u>human blood</u> (CAT +, OXI +, PZA 0, Growth 25 °C 0, COL R)

					Susceptibility		Growth		
	IAC	NO_3	URE	γGLU	NAL	CLTN	25°C	42°C	Remarks
Helicobacter spp. VA, BC		0	0	·	S (>16mm)	R	0	0	AlkP +
Helicobacter westmeadi	0	+	0	·	S	R	0	0	HIP +
Helicobacter cinaedi	0wk	+	0	0	S	V	0	0	AlkP 0
Helicobacter mainz	0	0	0	·	R	S (>16mm)	0	0	AlkP 0
Helicobacter fennelliae	+	0	0	0	S	S	0	0	
Flexispira rappini (CAT 0)	0	0	$+^R$	+	R	R	0	+	AlkP 0
Flexispira like (CAT +)	0	0	+	·	R	R	0	0	AlkP +
Helicobacter canis	+	0	0	+	S	R	0	+	AlkP +
Helicobacter pullorum	0	+	0	+	R	S	0	+	AlkP 0, COL S, Oxgall R,

IAC = Indoxyl Acetate D.T., HIP = Hippurate Hydrolysis D.T., NO_3 = Nitrate Reduction D.T., URE = Urease D.T., γGLU = Gamma-Glutamyl Aminopeptidase D.T., NAL = Nalidixan Neo-S, (S>16 mm R<16mm), CLTN = Cephalothin Neo-S (S>16mm R<16mm), AlkP = Alkaline Phosphatase D.T., CAT = catalase, $+^R$ = rapid positive, PZA = Pyrazinamidase D.T., COL = Colistin.

© Copyright Rosco Diagnostica A/S

3) Differentiation of *C. curvus*, *C. jejuni*, *Wolinella succinogenes*, and *Helicobacter pylori* (5)

All strains OXI + and MOT +.

	IAC	CAT	URE	NO_3
Campylobacter curvus	+ wk	0	0	+
Campylobacter jenuni	+	+	0	+
Wolinella succinogens	0	0	0	+
Helicobacter pylori	0	+	$+^R$	V

CAT = catalase, URE = Urease D.T., NO_3 = Nitrate Reduction D.T., OXI = Oxidase D.T., MOT = motility, $+^R$ = rapidly positive.

4) Differentiation of emerging *Campylobacter* spp., Arcobacter and Helicobacter from stools

Membrane filtration onto antibiotic-free media and incubation in an H_2-enriched microaerobic atmosphere at 37 °C is a simple and costeffective protocol for the isolation of all known Campylobacter, Arcobacter and Helicobacter spp. (6 Lastovica A.J. et al.).

	IAC	HIP	Growth McC	Rapid H_2S	NAL	CLIN	Remarks
C. jejuni subsp. *jejuni*	+	+	+	+/0	S/R	R	
C. jejuni subsp. *doylei*	+	+	0	0	S	S	NO_3 0
C. coli	+	0	+	0	S	R	
C. upsaliensis	+	0	0	0	S	S	
Arcobacter butzleri	+	0	+	0	V	R	Aerotolerant COL S
Arcobacter skirrowi	+	0	0	0	V	V	Aerotolerant COL S
*C. fetus *)*	0	0	+	0	R	S^R	
C. lari	0	0	0	+	R	R	
C. fennelliae	+	0	0	0	S	S	NO_3 0, COL R
H. cinaedi	0	0	0	0	S	V	NO_3 +, COL R
C. hyointestinalis	0	0	+	0	R	V	
C. concisus	0	0	0	0	V	V	
C. sputorum	0	0	+	+	R	S	

*)	CLTN	42 °C
C. fetus subsp. *fetus*	S	+
C. fetus subsp. *venerealis*	R	0

HIP = Hippurate Hydrolysis D.T., Growth McC = Growth in McConkey, NAL = Nalidixan Neo-S, CLTN = Cephalothin Neo-S, Col = colistin. NO_3 = Nitrate Reduction D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Indoxyl Acetate (Indoxyl acetate)	*Campylobacter jejuni* ATCC 33291	*E. faecalis* ATCC 51299

References

1) Mills C.K., Gherna R.L.: Hydrolysis of Indoxyl Acetate by Campylobacter Species. J. Clin. Microbiol. **25**, 1560-1561, 1987.
2) Sorlin P. et al: Recurrent Flexispira rappini bacteriemia in an adult patient undergoing hemodialysis: case report. J. Clin. Microbiol. **37**, 1319-23, 1999.
3) Weir S. et al: Recurrent bacteremia caused by a "Flexispira like" organism in a patient with X-linked agammaglobulinemia. J. Clin. Microbiol. **37**, 2439-45, 1999.
4) Weir S. et al: Un uncommon Helicobacter isolate from blood: evidence of a group of Helicobacter spp. pathogenic in AIDS patients. J. Clin. Microbiol. **37**, 2729-33, 1999.
5) Wetsch N.M. et al: Campylobacter curvus-associated hepatic abscesses: a case report. J. Clin. Microbiol. **44**, 1909-11, 2006.
6) Lastovica A.J. et al: Emerging campylobacter spp.: the tip of the iceberg. Clin. Microbiology Newsletter **28**, 49-55, 2006.

© Copyright Rosco Diagnostica A/S

3.23 LYSINE DECARBOXYLASE (LDC) ORNITHINE DECARBOXYLASE (ODC)

REF No. 56811
REF No. 57011

The diagnostic tablets are based on a modified conventional enzyme test between the active ingredient and a colour indicator. The active ingredient in Lysine Decaboxylase Diatabs is lysine and in Ornithine Decarboxylase Diatabs ornithine.
Decarboxylation of lysine by lysine decarboxylase yields cadaverine, while decarboxylation of ornithine yields putrescine. The production of these amines elevates the pH of the medium, changing the colour of the indicator from yellow to blue/violet (positive). If the organism does not produce the appropriate enzyme, the suspension remains acidic (yellow).

Procedure
Prepare a dense suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one diagnostic tablet and **3 drops of paraffin oil** and close the tube. The oil overlayer provides anaerobic conditions necessary to avoid false positive reactions. Incubate at 35-37 °C for **4 hours** or **up to 24 hours**.

Reading of the test
After 4 hours incubation:
Positive reaction: **Blue/violet**
Negative reaction: Yellow, green

After 18-24 hours incubation:
Positive reaction: **Strong violet**
Negative reaction: Yellow, green, grey or light blue

Results

1) **Both tests are well-known tests in the identification of Enterobacteriaceae and Vibrionaceae.**

2) **Ornithine Decarboxylase is used together with Indole and Urease in biotyping of *Haemophilus* spp.** (see page 49).

3) **Ornithine Decarboxylase is used for identification of *Staphylococcus lugdunensis***

	ODC	PYR	DEFRX	Remarks
Staphylococcus lugdunensis	+	+	R	(≤ 14 mm)
S. epidermidis/hominis	0^+	0	S	(≥ 16 mm)
Other CNS	0	V	R	

4) **Coryneform bacteria**

	LDC	ODC
Actinomyces neuii	70	+
Dermobacter hominis	+	+
Other fermentative coryneforms	0	0

ODC = Ornithine Decarboxylase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., DEFRX = Deferoxamine D.T., LDC = Lysine Decarboxylase D.T.

5) ***Burkholderia cepacia* complex (PYR 0, TRYP 0) (5)**
See under TRYPSIN, page 25.

© Copyright Rosco Diagnostica A/S

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Lysine Decarboxylase (LDC) (L-Lysine)	*K. pneumoniae* ATCC 13883	*Enterobacter cloacae* ATCC 13047
Ornithine Decarboxylase (ODC) (L-Ornithine HCl)	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 13883

References

1) Schnitzler N. et al: Staph. lugdunensis: report of a case of peritonitis and easy to perform screening strategy. J. Clin. Microbiol. **36**, 812-3, 1998.
2) Kahlmeter G. et al: S. lugdunensis orsakar inte bara endokardit, 1998.
3) Leung M.J. et al: Colony variation in Staph. lugdunensis. J. Clin. Microbiol. **36**, 3096-8, 1998.
4) Früh M. et al: Use of second-line biochemical and susceptibility tests for the differential identification of coryneform bacteria. Clin. Microbiol. Infect. **4**, 332-8, 1998.
5) Henry D.A.: Phenotypic methods for determining genomovar status of the Burkholderia cepacia complex. J. Clin. Microbiol. **39**, 1073-8, 2001.

© Copyright Rosco Diagnostica A/S

3.24 METRONIDAZOLE 5 µg (MTR.5)

REF No. 59711

Susceptibility to Metronidazole 5 µg can be used as a simple method **to screen for anaerobic bacteria**.

Procedure
The Metronidazole 5 µg diagnostic tablet (9 mm) is placed on an inoculated primary agar plate. The plate is incubated at 35-37 °C in anaerobic atmosphere for **24-48 hours**.

Apply one Metronidazole 5 µg tablet on the primary inoculum, using enriched blood agar. The tablet must be placed on the edge of the plate, otherwise growth of extremely susceptible organisms (fusobacteria) may be suppressed completely. Primary plates should be examined after incubation for 48 h and 5 days. Cell as well as colony morphology and smell are useful in the identification process of gram positive anaerobic cocci.

Reading of the test

	MTR.5
Anaerobic bacteria:	S (zone of inhibition ≥ 15 mm)
Microaerophilic bacteria	R (no zone of inhibition)
Aerobes:	R (no zone of inhibition)

Results

Gram positive **anaerobic** cocci (peptostreptococci) must be distinguished from **microaerophilic** organisms (streptococci, gemella, *Staph. saccharolyticus*).

1) Peptostreptococci (MTR.5 susceptible) and similar (most current clinical isolates)

	GLU	**α-GLU**	**IND**	**PRO**	**PYR**	**Alk P**	**SPS**	**Remarks**
P. anaerobius	+	+	0	+	0	0	S	(≥ 12 mm)
P. asaccharolyticus	0	0	+	0	0	+	R	
M. micros	0	0	0	+[0]		+	R/V	
F. magna	0	0	0	0	+	V	R	
P. stomatis	+	+	0	0	0	0	S	(≥ 15 mm)
A. vaginalis	+	V	0	0	0	V	R	LAP +
P. harei	0	0	0	0	0	0	R	

GLU = Glucose D.T., α-GLU = Alpha-Glucosidase D.T., IND = Indole D.T., PRO Proline Aminopeptidase D.T. , PYR = Pyrrolidonyl Aminopeptidase D.T., Alkaline Phosphatase D.T., SPS = SPS D.T.

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
Metronidazole 5 µg	*B. fragilis* ATCC 25285	*E. coli* ATCC 25922

References
1) Murdoch D.A.: Gram-positive anaerobic cocci. Clin. Microbiol. Reviews **11**, 81-120, 1998.
2) Yuli Song et al: Development of a flow chart for identification of gram-positive anaerobic cocci in the clinical laboratory. J. Clin. Microbiol. **45**, 512-516, 2007.

© Copyright Rosco Diagnostica A/S

3.25 METRONIDAZOLE 50 µg (MTR50)

REF No. 43611

Susceptibility to Metronidazole 50 µg and S.P.S. can be used as simple means to separate four major groups of vaginal bacteria that may be confused morphologically with *Gardnerella vaginalis*.

Procedure
Use the agar diffusion method with an inoculum equivalent to McFarland 0.5 on Mueller-Hinton II agar + 5% blood. Incubate in an atmosphere with 10% CO_2.

Results

1) *Gardnerella vaginalis*
CAT 0, OXI 0

	MTR50	SPS	α-GLU	ß-GLU	HIP	Remarks
Gardnerella vaginalis	S/R (≥12 mm S)	S (≥10 mm)	+	0	+	PRO +
G. vaginalis like organisms	R	R	.	.	$+^0$	
Lactobacilli	R	R	+	.	90	
Coryneforms	R	R	+	.	V	
Bifidobacteria	S	R	$+^0$	.	0	
Atopobium vaginae	R	R	0	0	.	PRO +, ADH +, LAP +, Vanco S

MTR50 = Metronidazole 50 µg D.T., SPS = SPS D.T., α-GLU = Alpha-Glucosidase D.T., ß-GLU = Beta-Glucosidase D.T., HIP = Hipurate Hydrolysis D.T.

Resistance of *G. vaginalis* to metronidazole may have arisen from widespread use of this drug to treat bacterial vaginosis (3). Resistance to metronidazole is now common among *G. vaginalis* isolates.

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
Metronidazole 50 µg	*G. vaginalis* ATCC 14018	*E. coli* ATCC 25922

References
1) Piot P.: Gardnerella, Streptobacillus, Spirillum, and Calymmatobacterium. pp. 483-487 in Manual of Clinical Microbiology, 5th ed., Balows A. et al (eds), ASM, 1991.
2) Bastida Vilá M.T.: Gardnerella vaginalis bacteremia in adult male. J. Clin. Microbio. Infect. Dis. **16**, 400-1, 1997.
3) McLean N.W. et al: Growth inhibition of metronidazole-susceptible and metronidazole-resistant strains of Gardnerella vaginalis by lactobacilli in vitro. Appl. Environm. Microbiol. **62**, 1089-2, 1996.

© Copyright Rosco Diagnostica A/S

3.26 NITRATE REDUCTION (NO_3)

REF No. 43711

Contain sodium molybdate and potassium nitrate.

Procedure 1
Prepare a dense "milky" suspension (at least McFarland No. 4) from the strain to be tested in 0.25 ml saline in a tube. Add one Nitrate Reduction tablet and close the tube. Incubate at 35-37 °C for **4 hours** or **18-24 hours**. After incubation **add 1 drop of N,N-Dimethyl-α-Naphthylamine sol.** and **1 drop Sulfanilic acid sol.** Read within 2 minutes.

Reading of the test
Positive reaction: **Red/pink**
Negative reaction: Colourless, light pink

Results

1) Most aerobes give a positive reaction. The following give a negative reaction:

	NO_3
Acinetobacter	0
Moraxella	0
Flavobacterium	0
some *Pseudomonas* spp.	0

2) Cocco-bacillary *Neisseria* spp. / Moraxella / Psychrobacter / Pasteurella (Oxidase +)

	CAT	GLU	NO_3	TRIB	COL	Pigment	Remarks
N. elongata subsp. *glycolytica*	0	$+^0$	0	0	S	+	
N. elongata subsp. *elongata*	+	0	0	0	S	+	
N. elongata subsp. *nitroreducens*	0	0	+	0	S	+	
N. weaveri	+	0	0	0	S	0	
N. bacilliformis (4)	0^+	0	$+^0$	$+^0$	S	+ wk	
Kingella denitrificans	0	+	+	0	R	0	
Kingella kingae	0	+	0	0	R	0	
Kingella potus	0	+	0	+	R	+	
Moraxella catarrhalis	+	0	$+^0$	+	V	0	
Psychrobacter spp.	+	+	V	V	.	0	URE +
Pasteurella spp.	+	+	+	.	S	0	IND $+^0$, ODC$+^0$, URE 0^+

CAT = catalase, GLU = Glucose D.T., NO_3 = Nitrate Reduction D.T., TRIB = Tributyrin D.T., COL = Colistin 10 µg D.T. (S ≥ 12 mm, R < 10 mm).

Procedure 2
When testing **anaerobes**, the tablet can also be placed on an inoculated plate, which is incubated for **24-48 hours**. After incubation **1 drop each of N,N-Dimethyl-α-Naphthylamine sol.** and **Sulfanilic acid sol.** is added to the tablet.

Reading of the test
A **pink or red** colour is interpreted as **positive** indicating reduction of nitrate to nitrite.

© Copyright Rosco Diagnostica A/S

Results

1) Among anaerobes the following give a positive reaction:

	NO_3
Bacteroides ureolyticus group	+
Veillonella	+
Propionibacterium acnes	+
Some *Clostridia* spp.	+
Eubacterium lentum	+
Bilophila wadsworthia	+
Wolinella/Campylobacter	+

2) Differentiation of Propionibacteria (Metro I/R)

	NO_3	IND	ß-XYL	CAT
Propionibacteria acnes	$+^0$	+	0	+
Propionibacteria avidum	0	0	+	+
Propionibacteria granulosum	0	0	0	+
P. propionicum (*Arachnia*)	+	0	0	0

NO_3 = Nitrate Reduction D.T., IND = Indole D.T., ß-XYL=Beta-Xylosidase D.T. and CAT = catalase.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Nitrate Reduction (Sodium Molybdate 40 µg, Potassium nitrate)	*E. coli* ATCC 25922	*S. saprophyticus* ATCC 15305

References

1) Wideman P.A., Citronbaum D.M., Sutter V.L.: Simple disk technique for detection of nitrate reduction by anaerobic bacteria. J. Clin. Microbiol. **5**, 315-319, 1977.
2) Foster G. et al: Staph. lutrae sp. nov., a new coagulase-positive species isolated from otters. Intl. J. Syst. Bacteriol. **47**, 724-6, 1997.
3) Funke G. et al: Clinical Microbiology of Coryneform bacteria. Clin. Microbiol. Reviews **10**, 125-159, 1997.
4) Lundgren B. et al: Two cases of endocarditis caused by Neisseria elongata subsp. nitroreducens and phenotypic differentiation from Kingella denitrificans. J. Clin. Microbiol. and Infect. **4**, 514-8, 1998.

© Copyright Rosco Diagnostica A/S

3.27 NOVOBIOCIN 5 µg (NOVO5) Neo-Sensitabs

REF No. 76312

May be used in the diagnostic work to differentiate **the *Staphylococcus saprophyticus* group** (causing urinary tract infections in young women) from **other coagulase negative staphylococci**. The *S. saprophyticus* group is resistant to Novobiocin 5 µg Neo-Sensitabs, while other staphylococci are sensitive. Use Mueller-Hinton II agar.
In anaerobe bacteriology Novobiocin 5 µg Neo-Sensitabs may be used as a presumptive test to differentiate ***Peptostreptococcus anaerobius/indolicus*** that are sensitive: (MIC <1.6 µg/ml), from other peptostreptocci that are resistant to novobiocin (MIC >25 µg/ml), i.e. zone size below 13 mm.

Procedure
For anaerobes use FAA + 5% blood or supplemented Brucella Blood agar with an inoculum equivalent to McFarland 0.5. Use current susceptibility testing media for staphylococci/ pediococci.

Results

1) *Staphylococcus saprohyticus* group

	McFarland 0.5 (Kirby- Bauer)		**Semi-confluent growth**
		Resistant (zone)	
S. saprophyticus, *Staphylococcus xylosus,* *Staphylococcus cohnii,* *S. cohnii* subsp. *urealyticum,* *Staphylococcus sciuri,* *Staphylococcus lentus*	< 13 mm		< 15 mm
		Sensitive (zone)	
Other staphylococci	≥ 14 mm		≥ 16 mm

2) *Staphylococcus hominis/epidermidis*

	NOVO5	**DEFRX**	**FOSFO**	**MSE**
S. hominis subs. *hominis*	S	S	R (<28 mm)	9
S. hominis subs. *novobiosepticus*	R	S	R (<28 mm)	9
S. epidermidis	S^R	S	S (>30 mm)	90
Other CNS	V	R	V	V

NOVO5 = Novobiocin 5 µg Neo-S, DEFRX = Deferoxamine D.T. (S ≥16mm, R ≤14mm), FOSFO = Fosfomycin Neo-S, MSE = Mannose D.T.

3) Anaerobes

	Sensitive (zone)
Peptostreptococcus anaerobius, P. indolicus, *P. heliotrinreducens,*	≥ 14 mm
	Resistant (zone)
P. asaccharolytica, *F .magna, M. micros,* *A. prevotii, A. tetradius*	< 13 mm

© Copyright Rosco Diagnostica A/S

4) Pediococci

	NOVO5	MAL
Pediococcus acidilactici	S	0
Pediococcus pentosaceus	R	+

NOVO5 = Novobiocin 5 µg Neo-S and MAL = Maltose D.T.

References

1) Wren M.W.D., Eldon C.P., Dakin G.H.: Novobiocin and the differentiation of peptococci and peptostreptococci. J. Clin. Path. **30**, 620-622, 1977.
2) Casals J.B., Pringler N.: Identification of staphylococci using a combination of chromogenic substrates and sensitivities towards Furazolidone, Novobiocin and Colistin. Workshop on Pathogenesis of Wound and Biomaterial-Associated Infections, Lund University, 1989.
3) Wegener H.C.: Diagnostic value of phage typing, antibiogram typing, and plasmid profiling of Staph. hyicus from piglets with exudative epidermitis J.Vet.Med. 13-20, 1993.
4) Devriese L.A.: A simple identification scheme for coagulase negative staphylococci from bovine mastitis. Research in Vet. Science **57**, 240-4, 1994.
5) Weinstein M.P. et al: Clinical importance of identifying CNS isolated from blood cultures: evaluation of Microscan panels versus a conventional Reference Method. J. Clin. Microbiol. **36**, 2089-92, 1998.

© Copyright Rosco Diagnostica A/S

3.28 O/129 (Vibriostaticum) (O/129)

REF No. 45411

Vibrios are sensitive to the vibriostatic agent O/129 (2,4-diamino 6,7 di-isopropyl pteridine). The diffusible amount is 150 µg per tablet. The O/129 is useful for differentiation of **Vibrios** from **Enterobacteriaceae** and **Aeromonas**. O/129 is also useful in the differentiation of corynebacteria.

Procedure
A plate of Oxoid Blood Agar Base (CM271) containing 0.5% NaCl is seeded with the culture under test and the O/129 150 µg diagnostic tablet is applied. The plates are incubated for **24 hours** before reading sensitivity.

If commercial sensitivity agar is used instead of CM271, many of the marine vibrio strains will not grow, but in addition many enterobacteria will show a degree of sensitivity to O/129.

Strains with acquired resistance against trimethoprim will also be resistant to O/129.

Reading of the test
Sensitive: ≥16 mm
Resistant: <16 mm

Results

1a) Differentiation of *Aeromonas* spp.

	ODC	LDC	ADH	ARA	TRYP	Remarks
Aeromonas hydrophilia	0	+	+	+	27	
Aeromonas caviae	0	0	+	+	+	
Aeromonas veronii (*sobria*)	0	+	+	0	$+^{0}$	
Aeromonas (veronii)	+	+	0	0	0	URE +

1b) Vibrio/Aeromonas/Plesiomonas/Photobacterium

Most strains are: OXI +, GLU +

	O/129	ADH	ODC	MAN
Vibrio spp.	S	0^{+}	$+^{0}$	+
Plesiomonas shigelloides	S	+	+	0
Aeromonas	R	$+^{0}$	0^{+}	+
Photobacterium spp.	S	+	0	0

Note that strains showing resistance to trimethoprim or trhimethoprim + sulfa connot reliably be tested with O/129.

ODC = Ornithine Decarboxylase D.T., LDC = Lysine Decarboxylase D.T., ADH = Arginine Dihydrolase D.T., ARA = Arabinose D.T., TRYP = Trypsin D.T., MAN = Mannitol D.T.

1c) Vibrio/Enterobacteriaceae/Pasteurellaceae

	MOT	OXI	Alk P	O/129	High salt
Vibrio spp.	+	+	V	S	Growth
Enterobacteriaceae	+	0	0	R	V
Pasteurellaceae	0	+	+	S	No growth

MOT = motility, OXI = Oxidase D.T., Alk P = Alkaline Phosphatase D.T., High salt = medium with high salt content.

© Copyright Rosco Diagnostica A/S

2) Corynebacteria nonlipophilic, fermentative

	O/129	NAG	LAP	MAL	AMP	α-GLU
Corynebacterium striatum	S	0	82	0	S (≥ 23 mm)	0
C. minutissimum	S	89	+	+	S	0
C. amycolatum (F-2)	R	0	0	80	R^S (< 20 mm)	0^+
Corynebacterium xerosis	S	.	$+^0$	+	R^S	$+^0$

3) Corynebacteria

	O/129	PZA
Corynebacterium diphtheriae	S	0
Corynebacterium imitans	R	wk
Corynebacterium striatum	S	+

O/129 = O/129 150 µg D.T., NAG = Beta-N-Acetylglucosaminidase D.T., LAP = Leucine Aminopeptidase D.T., MAL = Maltose D.T., PZA = Pyrazinamidase D.T., AMP = Ampicillin 33 µg Neo-S, ADH = Arginine Dihydrolase D.T., OCT = Ornithine Decarboxylase D.T., α-GLU = Alpha-Glucosidase D.T.,

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
O/129 150 µg (2,4-Diamino-6,7-diisopropylpteridine phosphate salt)	*Kocuria rhizophila* ATCC 9341	*E. coli* ATCC 25922

References

1) Lee J.V.: Identification of Aeromonas, Vibrio and related organisms, pp. 152-165 in Identification methods for microbiologists. Skinner F.A., Lovelock D.W. (Ed.s.), Acad. Press London, N.Y. 1979.
2) Baumann P., Schubert H.W.: Vibrionaceae, page 535 in Bergey's Ma nual of Systematic Bacteriology, vol. 1, 1984.
3) Dalgaard P.: Qualitative and quantitative characterization of spoilage bacteria from packed fish. Intl. J. Food Microbiol. **26**, 319-333, 1995.
4) Abbott S.L. et al: Misidentification of unusual Aeromonas spp. as members of the genus Vibrio: a continuing problem. J. Clin. Microbiol. **36**, 1103-4, 1998.
5) Früh M. et al: Use of second-line biochemical and susceptibility tests for the differential identification of coryneform bacteria. Clin. Microbio. Infect. **4**, 332-8, 1998.
6) Renaud F.N.R.: Differentiation of Corynebacterium amycolatum, C. minutissimum and C. striatum by carbon substrate assimilation tests. J. Clin. Microbiol. **36,** 3698-3702, 1998.
7) Abbot S.L. et al: The genus Aeromonas: biochemical characteristics, atypical reactions and phenotypic identification schemes. J. Clin. Microbiol. **41**, 2348-57, 2003.

© Copyright Rosco Diagnostica A/S

3.29 OPTOCHIN (OPT) OXGALL (OXG)

REF No. 44211
REF No. 44311

Optochin is an agent capable of inhibiting growth of pneumococci, but not alpha-streptococci or other streptococci. Optochin Diatabs contain 10 µg of diffusible optochin and are useful for the presumptive identification of **pneumococci**.
Oxgall is useful being a substitute of the bile solubility test; each tablet contains 1000 µg diffusible oxgall.

Procedure
Pneumococci (incubated in an atmosphere containing CO_2 on an agar with blood) show an inhibition zone ≥ 18 mm around Optochin diagnostic tablets, while streptococci show inhibition zones of < 16 mm. In the event of inhibition zones of 16-17 mm, the test is repeated with optimum inoculum (McFarland 0.5).

Pneumococci incubated aerobically show a zone of inhibition ≥ 20 mm, but the preferred method is CO_2 atmosphere (6).
The optimal blood agar is TSA with 5 % sheep blood.
With Oxgall, pneumococci show a zone of ≥ 19 mm.

Reading of the test

	Optochin	Oxgall
CO_2 atmosphere:		
Pneumococci:	≥ 18 mm	≥ 19 mm
Streptococci :	< 16 mm	≤ 17 mm
Aerobe atmosphere:		
Pneumococci:	≥ 20 mm	
Streptococci:	< 18 mm	

Results

1) Differentiation of non-beta-haemolytic streptococci

	OPT	BE	PYR	HIP	OXG	α-GAL	ADH	Remarks
S. pneumoniae	S	0	0	0	S	+	+	TRE +
Group B strep (non ß-haem)	R	0	0	+	R	·	·	
S. bovis	R	+	0	·	R	·	·	
Viridans streptococci	R	0^+	0^+	0^+	R	·	·	
NVS (*Abiotrophia* spp.)	R	0	+	V	·	·	·	
S. mitis	R/S	0	0	0	R	·	0	TRE 0
S. pseudopneumoniae	R/S	0	0	0	R	0	+	TET R^S, ERY R^S, TRE V

OPT = Optochin D.T., BE = Bile Esculin D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., HIP = Hippurate Hydrolysis D.T., NVS = Nutritionally variant streptococci, OXG = Oxgall D.T. (S ≥ 19 mm, R ≤ 17 mm), α-GAL = Alpha-Galactosidase D.T., ADH = Arginine Dihydrolase D.T.,.

2) Differentiation of *S. pneumoniae, S. pseudopneumoniae* and *S. mitis/oralis* group (7,8)

	OPT	ADH	1% Deox	0.1% Deox
S. pneumoniae	S	+	S	S
S. pseudopneumoniae	R^S	+	R	S
S. mitis/oralis group	R^S	0^+	R	S

OPT = Optochin D.T., ADH = Arginine Dihydrolase D.T., 1 % Deox = 1 % sodium deoxycholate lysis, 0.1 % Deox = 0.1 % sodium deoxycholate lysis.

© Copyright Rosco Diagnostica A/S

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
Optochin 10 µg (Ethylhydrocuprein HCl)	*S. pneumoniae* ATCC 49619	*S. bovis* ATCC 15351
Oxgall 1000 µg (Oxgall)	*S. aureus* ATCC 25923	*B. fragilis* ATCC 25285

References

1) Ragsdaler R.A., Sanford J.P.: Interfering effect of incubation in carbon dioxide on the identification of pneumococci by optochin discs. Appl. Microbiol. **22**, 854-855, 1971.
2) Gardam M.A.: Optochin revisited: defining the optimal type of blood agar for presumptive identification of Strept. pneumoniae. J. Clin. Microbiol. **36**, 833-4, 1998.
3) Ruoff K.L.: Streptococci. Manual Clinical Microbiology 6th Ed., 299-307, 1995.
4) Kellog J.A. et al: Identification of Strept. pneumoniae revisited. J. Clin. Microbiol. **39**, 3373-5, 2001.
5) Christensen A. et al: Pneumococci and bile solubility. Clin. Microbiol. Infect. **6**, Suppl. 1, 163, 2000.
6) Arbique J.C. et al: Accuracy of phenotypic and genotypic testing for identification of S. pneumoniae and description of S. pseudopneumoniae sp. nov. JCM **42**, 4686-96, 2004.
7) Keith E.R. et al: Characteristics of S. pseudopneumoniae isolated from purulent sputum samples. J. Clin. Microbiol. **44**, 923-7, 2006.
8) Balsalobre at al: Molecular characterization of disease-associated streptococci of the mitis group that are optochin susceptible. J. Clin. Microbiol. **44**, 4163-71, 2006.

© Copyright Rosco Diagnostica A/S

3.30 OXIDASE (OXI)

REF No. 45711

The oxidase test is useful in the presumptive identification of Neisseria as well as for miscellaneous gram-negative bacteria (Non-Fermenters, Vibrionaceae, Campylobacter, etc.).

Oxidase Diatabs contain the substrate NNN'N'-tetramethyl-p-phenylenediamine 2 HCl, which is very sensitive.

Procedure
Lay a thick filter paper in an empty petri dish and place an Oxidase diagnostic tablet on it. Add **one drop of saline on top of the tablet**, wait 60 seconds and add **another drop of saline on top of the tablet**.

When the filter paper around the tablet is wet**, the colony is immediately smeared** onto the wet filter paper approx. 3-8 mm apart from the edge of the tablet using a plastic or platinum loop (Nichrome and iron containing wires give false positive reactions).

Reading of the test
Make the reading **within 2 min.** of smearing the filter paper. The colony turns **blue/purple** when the strain is **oxidase positive**. Use a positive control in cases of weak positive reactions.

Results

Among the oxidase positive microorganisms are:

Neisseria	Aeromonas	Pasteurella
Vibrio	most *Pseudomonas* spp.	Flavobacterium
Alcaligenes	Moraxella	Campylobacter
Plesiomonas.		

Among the oxidase negative are:

Staphylococci	streptococci	anaerobes
Enterobacteriaceae	Acinetobacter	*Stenot. maltophilia*
Haemophilus.		

Differentiation of Anaerobispirillum from Campylobacter:

	OXI	Ery	CAT
Campylobacter spp.	$+^0$	S (≥ 30 mm)	$+^0$
Anaerobispirillum spp. (succiniproducens)	0	R (≤ 23 mm)	0

OXI = Oxidase D.T., Ery = Erythromycin Neo-S, CAT = catalase.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Oxidase (Tetramethyl-p-phenylenediamine)	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922

References
1) Gadberry J.L., Clemmons K., Drumm K.: Evaluation of methods to detect oxidase activity in the genus Pasteurella. J. Clin. Microbiol. **12**, 220- 225, 1980.
2) Tee W. et al: Three cases of Anaerobispirillum succiniproducens bacteremia confirmed by 16 S + RNA Gene sequencing. J. Clin. Microbiol. **36**, 1209-13, 1998.

© Copyright Rosco Diagnostica A/S

3.31 POLYMYXINS 150 µg (CO150) Neo-Sensitabs

REF No. 77512

Polymyxins 150 µg (Colistin) Neo-Sensitabs are useful in identification of staphylococci. ***S. aureus*** (zones ≤ 12 mm) is the most resistant species, but some strains of *S. epidermidis*, *S. hyicus* and a few strains of *S. lugdunensis* may also show small zones of inhibition (≥ 13 mm).

Procedure
Sensitivity testing of staphylococci is performed on Mueller-Hinton II agar without blood using McFarland 0.5 inoculum. Danish Blood Agar may be used with semiconfluent growth.. Polymyxins 150 µg Neo-Sensitabs may be added to the routine antibiogram. Incubate at 35 °C overnight.

Results

1) Staphylococci

	McFarland 0.5 (Kirby- Bauer)		**Semi-confluent growth**
		Resistant	
S. aureus	≤ 12 mm		≤ 15 mm
		Sensitive	
Other staphylococci	≥ 14 mm		≥ 16 mm

2) Non fermenters

	Poly
Shewanella algae	R (< 18 mm)
Shewanella putrefaciens	S (≥ 20 mm)

3) Cocco-bacillary *Neisseria* spp. / Moraxella / Psychrobacter / Pasteurella (Oxidase +)

	CAT	**GLU**	**NO_3**	**TRIB**	**POLY**	**Pigment**	**Remarks**
N. elongata subsp. *clycolytica*	0	$+^0$	0	0	S	+	
N. elongata subsp. *elongata*	+	0	0	0	S	+	
N. elongata subsp. *nitroreducens*	0	0	+	0	S	+	
N. weaveri	+	0	0	0	S	0	
N. bacilliformis (7)	0^+	0	$+^0$	$+^0$	S	+ wk	
Kingella denitrificans	0	+	+	0	R	0	
Kingella kingae	0	+	0	0	R	0	
Kingella potus	0	+	0	+	R	+	
Moraxella catarrhalis	+	0	$+^0$	+	V	0	
Psychrobacter spp.	+	+	V	V	.	0	URE +
Pasteurella spp.	+	+	+	.	S	0	IND $+^0$, ODC$+^0$, URE 0^+

CAT = catalase, GLU = Glucose D.T., NO_3 = Nitrate Reduction D.T., TRIB = Tributyrin D.T., POLY = Polymyxins 150 µg Neo-S (S ≥ 20 mm, R < 18 m).

Quality Control (McF 0.5)

NEO-SENSITABS	Code	***E. coli*** **ATCC 25922**	***Ps. aeruginosa*** **ATCC 27853**
Polymyxins 150 µg	CO150	19-24 mm	20-25 mm

© Copyright Rosco Diagnostica A/S

References

1) Heltberg O., Bruun B.: Polymyxin susceptibility in staphylococci differentiating coagulase positive and coagulase negative strains. Acta path. microbiol. immunol. Scand., Sect. B, **91**, 157-161, 1983.
2) Heltberg O., Bruun B.: Recognition of coagulase negative Staph. aureus strains by primary polymyxin usceptibility testing. Acta path. microbiol. immunol. Scand., Sect. B, **92**, 115-118, 1984.
3) Casals J.B., Pringler N.: The value og 3 tests in the identification of staphylococci: Pyrrolidonyl aminopeptidase (PYR) and Susceptibility towards Polymyxins and Furazolidone. Staphylococci Symposium, Society for Applied Bacteriology, Edinburgh, July 1989.
4) Casals J.B., Pringler N.: Identification of staphylococci using a combination of chromogenic substrates and sensitivities towards Furazolidone, Novobiocin and Colistin. Workshop on Pathogenesis of Wound and Biomaterial-Associated Infections, Lund University, 1989.
5) Holt H.M. et al: For infections with Shewanella alga: a bacteriologic, clinical and epidemiological study of 67 cases. Clin. Microbiol. Infect. **3**, 329-333, 1997.
6) Lundgren B. et al: Two cases of endocarditis caused by Neisseria elongata subsp. nitroreducens and phenotypic differentiation from Kingella denitrificans. Clin. Microbiol. Infect. **4**, 514-8, 1998.
7) Xiang Y. Han et al: Neisseria bacilliformis sp. nov. isolated from human infections. J. Clin. Microbiol. **44**, 474-79, 2006.

© Copyright Rosco Diagnostica A/S

3.32 PORPHYRIN (d-Ala) (ALA)

REF No. 57321

Contain delta-aminolevulinic acid for the detection of hemin (X-factor) requirement in the **differentiation of *Haemophilus influenzae* from *Haemophilus parainfluenzae***. This enzymatic test is rapid and independent of several factors affecting the usual tests for growth factor requirements (e.g. presence of X-or V-factor in the test medium, "carry over" of X-factor with the inoculum from chocolate agar, lack of other essential nutrients in the test medium).

Principle of the Test
Haemophilus parainfluenzae does not require hemin (X-factor) for growth because it possesses enzymes for the biosynthesis of heme (Fig. 1). When supplied with delta-aminolevulinic acid, *Haemophilus parainfluenzae* strains synthetize porphobilinogen and porphyrins, which are detected in the test.

Porphyrins show characteristic **red fluorescence** when exposed to long wave UV-light (360 nm). Porphobilinogen that contains a pyrrole ring produces a **red colour with Kovacs' reagent** (92031) (in the lower water phase).

Kilian (1974) tested 134 Haemophilus strains and found a perfect agreement between the two methods of reading.

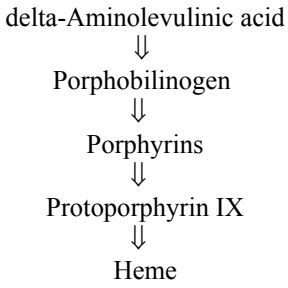


Fig. 1. Main steps of the heme biosynthesis.

Haemophilus influenzae that requires hemin for growth lacks the enzymes for heme synthesis and consequently does neither produce porphyrins nor porphobilinogen from delta-aminolevulinic acid (negative reaction).

Procedure
Prepare a dense bacterial suspension (at least McFarland No. 4) of fresh colonies (18-24 hours) of the haemophilus strain to be tested in 0.25 ml saline in a tube. Add one Porphyrin (ALA) diagnostic tablet and close the tube. Incubate at 35-37 °C for **4-6 hours** or in case of negative or doubtful reactions for **up to 24 hours**.

Reading of the test
The test can be read in two ways:

a) Add **4 drops of Kovacs' reagent** (92031), shake and wait for up to 10 minutes.

Positive reaction:	**Red/pink** colour in the **lower water phase**
The strain does not require X-factor:	***Haemophilus parainfluenzae***
Negative reaction:	**Colourless** water phase
The strain requires X-factor:	***Haemophilus influenzae***

After the addition of Kovacs' reagent the tube cannot be reincubated for re-reading. For rapid results it is advisable to incubate two tubes for each strain: one for addition of reagent after 4 hours, and another for later confirmation (re-incubation), if a negative test result is obtained.

© Copyright Rosco Diagnostica A/S

b) Expose to long wave UV light, 360 nm (Wood's lamp).

Positive reaction: **Red fluorescence**
The test strain does not require X-factor: ***Haemophilus parainfluenzae***

Negative reaction: **No red fluorescence**
The test strain requires X-factor: ***Haemophilus influenzae, Haemophilus haemolyticus***

In case of doubtful or negative reactions, the tube should **be re-incubated for 18-24 hours**.

Other haemophilus

	ALA
H. ducreyi (usualliy genital sources)	0
H. aegyptius (conjunctivitis)	0
H. haemolyticus (oral flora)	0
Aggregatibacter aphrophilus	+ or 0
Aggregatibacter segnis	0

Other bacteria

	ALA (Kovacs')
Staphylococci (CAT +)	+
Staphylococci (CAT 0)	+
Streptococci	0
Aerococci	0
Rothia mucilaginosa	+

ALA = Porphyrin D.T.

1) Differentiation of *Aggregatibacter* spp. (ALA +, NO_3 +, Alk P +, non-haem)

	ALA	**CAT**	**ONPG**	**SUC**	**TRE**	**γ-GLU**
A. actinomycetemcomitans	+	+	0	0		+
A. aphrophilus	+	0	+	+	+	+
A. segnis	+	V	V	+	0	0
Haemophilus parainfluenzae	+	.	.	+	0	0

CAT = Catalase, ONPG = ONPG D.T. SUC = Sucrose D.T., TRE = Trehalose D.T. , γ-GLU = Gamma-Glutamyl Aminopeptidase D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Porphyrin (d-Ala) (d-Aminolevulinic acid)	*H. parainfluenzae* ATCC 7901	*H. influenzae* ATCC 49247

References

1) Kilian M.: A Rapid Method for the Differentiation of Haemophilus strains. Acta Path. Microbiol. Scand. Sect. B **82**, 835-842, 1974.
2) Kilian M.: Haemophilus. Ch. 25 in Manual of Clinical Microbiology, 3rd ed. Lennette E.H. et al. (Eds.). American Society for Microbiology, Washington D.C., 1980.
3) Kilian M., Sørensen I., Frederiksen W.: Biochemical Characteristics of 130 Recent Isolates from Haemophilus influenzae Meningitis, J. Clin. Microbiol. **9**, 409-412, 1979.
4) Tebbutt, G.M.: Evaluation of some methods for the Laboratory identification of Haemophilus influenzae. J. Clin. Pathol. **36**, 991-995, 1983.
5) Wong J.D.: Porphyrin test as an alternative to benzidine test for detecting cytochromes in catalase negative gram-positive cocci. J. Clin. Microbiol. **25**, 2006-7, 1987.
6) Munson E. et al: Comparison of porphirin-based, growth factor based and biochemical based testing methods for identification of Haemophilus influenzae. Eur. J. Clin. Microbiol. Infect. Dis. **21**, 196-203, 2002.

© Copyright Rosco Diagnostica A/S

7) Nørskov-Lauridsen N., Kilian M.: Reclassification of Actinobact. actinomycetemcomitans, H. aphrophilus, H. paraphrophilus, H. segnis as Aggregatibacter actinomycetemcomitans gen. nov. comb. nov., A. aphrophilus comb. nov. and A. segnis com. nov. and emended description of A. aphrophilus to include V-factor dependent and V-factor independent isolates. IJSEM **56**, 2135-2146, 2006.

© Copyright Rosco Diagnostica A/S

3.33 PS. AERUGINOSA SCREEN (PSAER)

REF No. 59311

Keeven and DeCicco (1989) found that 1,10-phenanthroline has a high selective specificity for *Pseudomonas aeruginosa*.

Ps. aeruginosa Screen Diatabs contain 80 µg diffusible amount per tablet and are useful for the presumptive identification of *Pseudomonas aeruginosa*.

Procedure
Place one Ps. aeruginosa Screen Diagnostic Tablet on an inoculated plate (Mueller-Hinton agar or similar) for sensitivity testing. Incubate at 35-37 °C for **18-24 hours**.

Read the diameter of the inhibition zone in mm. Measure **only the clear zone** with no growth.

Reading of the test

	PSAER	
	10^8 CFU/ml Confluent growth (Kirby-Bauer)	**10^5-10^6 CFU/ml Semi-confluent growth**
Pseudomonas aeruginosa	≤ 14 mm	≤ 16 mm
Other Pseudomonas species, non-fermenters, and Enterobacteriaceae	≥ 18 mm	≥ 20 mm

Results

1) Strains from cystic fibrosis patients

	PSAER	**COL**	**PYR**	**TRYP**	**TRIB**	**Remarks**
Ps. aeruginosa	R	S^R	$+^0$	+	$+^0$	
Burk. cepacia complex	S	R^S	0	0	+	ADH 0, GLU + Genta R
Achr. xylosoxidans	S	V	+	0	0	Aminoglyc R, Quinolones R
St. maltophilia	S	V	0	+	+	
Inquilinus limosus	S	R	+	+	.	res
Pandoraea spp.	S	R	·	0	$+^0$	Meropenem R, URE +, Alk P +, Genta/Tobra R, LAP +, MOT +, CAT +

COL = Colistin 10 µg D.T. (S ≥ 13 mm, R ≤ 10 mm), PYR = Pyrrolidonyl Aminopeptidase D.T., TRYP = Trypsin D.T., S^R = most strains are S, R^S = most strains are R, res = multiresistant, TRIB = Tributyring D.T.

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
Ps. aeruginosa Screen 80 µg (1,10 Phenanthroline)	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853

References
1) Keeven J.K., De Cicco B.T.: Selective medium for Pseudomonas aeruginosa that uses 1,10-Phenanthroline as the selective agent. Appl. Environm. Microbiol. **55**, 3231-3233, 1989.
2) Pringler N., Casals J.B.: Identification of Pseudomonas aeruginosa with Ps. aeruginosa Screen Diagnostic Tablets, 5th European Congress of Clinical Microbiology and Infectious Diseases, Oslo, Sep., 1991.
3) Fijita S. et al: Identification of Ps. aeruginosa by using a disk of phenanthroline and C-390 and by cell agglutination testing with monoclonal antibodies. J.Clin. Microbiol. **30**, 2728-9, 1992.

© Copyright Rosco Diagnostica A/S

3.34 PYRAZINAMIDASE (PZA)

REF No. 59811

Test to differentiate pathogenic corynebacteria (negative reaction) from other corynebacteria (positive reaction). The enzyme pyrazinamidase catalyzes the hydrolysis of pyrazinamide into pyrazinoic acid and ammonia. Also useful in the differentiation of *Yersinia enterocolitica* serotypes.

Procedure
Make a turbid, "milky" suspension equivalent to at least McFarland No. 8 (Corynebacteria) or No. 4 (Yersinia) of the test strain from an agar plate culture in 0.25 ml distilled water in a tube. Add one PZA Diatabs and close the tube. Incubate for **4 hours** or **over-night** at 35-37 °C.

Reading of the test
After incubation add one drop of ferrous ammonium sulphate solution 5% w/v in purified water (freshly prepared or stored at -20°C).

Positive reaction: **Orange, red**
Negative reaction: Colourless, light yellow

Results

1) Corynebacteria

The pathogenic species *C. diphtheriae*, *C. pseudotuberculosis* and *C. ulcerans* give a negative reaction, while other corynebacteria give a positive or variable reaction.

2) *Yersinia enterocolitica* (5)

	PZA	SAL	ESC
Yersinia enterocolitica (pathogenic serotype)	0	0	0
Yersinia enterocolitica non-pathogenic	+	+	+
Yersinia spp.	+	V	V

PZA = Pyrazinamidase D.T., SAL = Salicin D.T., ESC = Esculin Hydrolysis D.T. All tests performed at **25 °C**.

3) *Yersinia enterocolitica* and *Y. enterocolotica*-like nontipeable species (6)

	COL on CIN agar	ESC	SAL	PZA
Yersinia enterocolitica	No ground glass appearance	V	V	0^+
Yersinia enterocolitica-like	Erose edges			
a) *Y. mollaretti*	and ground glass	0	0	+
b) Y. bercovieri	appearnce	0	0	+

Col on CIN agar = colony morphology on CIN–agar. All test at 25 °C.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Pyrazinamidase (Pyrazinamide)	*E. coli* ATCC 25922	*S. aureus* ATCC 25923

© Copyright Rosco Diagnostica A/S

References

1) Colman G., Weaver E., Efstratiou A.: Screening tests for pathogenic corynebacteria. J. Clin. Pathol. **45**, 46-48, 1992.
2) Farmer III J.J. et al: Pyrazinamidase, CR-MOX Agar, Salicin fermentation Esculin hydrolysis and D-xylose fermentation for identifying pathogenic serotypes of Yers. enterocolitica. J. Clin. Microbiol. **30**, 2589-94, 1992.
3) Efstratiou A., Sesardic D., Hoy C.S., Sangrador A., Cookson B.D.: Microbiological Diagnosis of Diphtheria. Poster 448, 6th European Congress of Clinical Microbiology and Infectious Diseases, Seville, Spain, March, 1993.
4) Efstratiou A.: Laboratory Diagnosis of Diphtheria (European Region WHO). Pyrazinamidase Test and Rosco Identification System, pages 9-10, 37-42, 1994.
5) Chiesa C. et al: Identification of pathogenic serotypes of Yersinia enterocolitica. J.Clin. Microbiol. **31**, 2248-50, 1993.
6) Hallanvûo S. et al: Simplified phenotypic scheme evaluated by 165 rRNA sequencing for differentiation between Y. enterocolytica and Y. enterocolytica-like spp. JCM **44**, 1077-80, 2006.

© Copyright Rosco Diagnostica A/S

3.35 S.P.S. (SPS)

REF No. 44611

SPS Diatabs contain 1 mg diffusible amount of sodium polyanethol sulfonate per tablet and are useful for identification of ***Peptostreptococcus anaerobius*** and ***Gardnerella vaginalis***.

Procedure
SPS diagnostic tablets are placed on inoculated blood agar plates (inoculum equivalent to McFarland 0.5 - confluent growth) before incubation.

1) ***Peptostreptococcus anaerobius*** is sensitive to SPS and a zone of inhibition around the diagnostic tablet is produced (≥12 mm). Incubate anaerobically for 48 hours at 35-37 °C.

   Other *Peptostreptococcus* species are resistant to SPS (no zone of inhibition).

	SPS	GLU	α-GLU	IND	PRO	PYR	Alk P	Remarks
P. anaerobius	S (≥ 12 mm)	+	+	0	+	0	0	
P. stomatis	S (≥ 15 mm)	+	+	0	0	0	0	
P. asaccharolyticus	R	0	0	$+^0$	0	0	+	
M. micros	V	0	0	0	$+^0$	+	+	
F. magna	R	0	0	0	0	+	V	
A. vaginalis	R	+	V	0	0	0	V	LAP +
P. harei	R	0	0	0	0	0	0	

SPS = SPS D.T., GLU = Glucose D.T., α-GLU = Alpha-Glucosidase D.T., IND = Indole D.T., PRO = Proline D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., Alk P = Alkaline Phosphatase D.T.

Use FAA + 5% blood or Brucella Blood Agar **and incubate in anaerobic atmosphere**.

2) ***Gardnerella vaginalis*** is sensitive to SPS and shows an inhibition zone of ≥10 mm.

   Lactobacillus spp., *Corynebacterium* spp., *Bifidobacterium* spp., and vaginal streptococci are resistant.

	SPS	HIP	α-GLU	ß-GLU	Remarks
Gardnerella vaginalis	S (≥ 10 mm)	+	+	0	CAT 0, OXI 0, PRO +
Vag. lactobacilli	R	V	+	.	
Vag. corynebacteria	R	V	V	.	
Bifidobacterium	R	0	+	.	
Atopobium vaginae	R	.	0	0	PRO +, ADH +, LAP +, Metro R, Vanco S

HIP = Hippurate hydrolysis D.T., ß-GLU = Beta-Glucosidase D.T.

Use Mueller-Hinton II agar + 5% blood and **incubate in an atmosphere with 5-10% CO_2**.

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
S.P.S. 1000 µg (Sodium polyanethol sulfonate)	*G. vaginalis* ATCC 14018	*Kocuria rhizophila* ATCC 9341

© Copyright Rosco Diagnostica A/S

References

1) Graves M.H., Morello J.A., Kocka F.E.: Sodium polyanetholsulfonate sensitivity of anaerobic cocci. Appl. Microbiol. **27**, 1131-1133, 1974.
2) Reimer L.G., Reller L.B.: Use of a Sodium Polyanetholesulfonate Disk for the Identification of Gardnerella vaginalis. J. Clin. Microbiol. **21**, 146-149, 1985.
3) Bastida Vilá M.T et al: Gardnerella vaginalis bacteremia in an adult male. Eur. J. Clin. Microbiol. Infect. Dis. **16**, 400-1, 1997.
4) Murdoch D.A.: Gram-positive anaerobic cocci. Clin. Microbiology Reviews **11**, 81-120,1998.
5) Yuli Song et al: Development of a flow chart for identification of gram-positive anaerobic cocci in the clinical laboratory. J. Clin. Microbiol. **45**, 512-516, 2007.

© Copyright Rosco Diagnostica A/S

3.36 SUGAR FERMENTATION Tests (SFT)

Diatabs for sugar fermentation contain the specific sugar substrate together with a weak buffer and an indicator (phenol red), which changes colour from red to **yellow** in case of a **positive** reaction.

Range
The range of sugar fermentation tests comprises:

Diatabs	Code	REF No.
Adonitol	(ADO)	(52011)
l-Arabinose	(ARA)	(52121)
Cellobiose	(CEL)	(non-stock)
Dulcitol	(DUL)	(non-stock)
Fructose	(FRU)	(non-stock)
Galactose	(GAL)	(non-stock)
Glucose	(GLU)	(52611)
Inositol	(INO)	(non-stock)
Inulin	(INU)	(52711)
Lactose	(LAC)	(52811)
Maltose	(MAL)	(52911)
Mannitol	(MAN)	(53011)
Mannose	(MSE)	(53111)
Melibiose	(MEL)	(53211)
Raffinose	(RAF)	(53311)
l-Rhamnose	(RHAM)	(53411)
Ribose	(RIB)	(non-stock)
Salicin	(SAL)	(non-stock)
Sorbitol	(SOR)	(53711)
Sucrose	(SUC)	(53811)
Trehalose	(TRE)	(53911)
d-Xylose	(XYL)	(54021)

Procedure
Please note: Colonies should be obtained from bacteria grown on sugarfree media, such as TSA agar.
Prepare a dense bacterial suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one sugar diagnostic tablet and close the tube. Incubate at 35-37 °C for **4 hours** or **overnight**.

Positive reaction: **Yellow**, yellow-orange (4 hours), **yellow** (overnight)
Negative reaction: Red, orange-red

For identification of pathogenic Neisseria we recommend the use of enzymatic tests (Gamma Glutamyl aminopeptidase (46711), Tributyrin (48821), ONPG (50311) and the Superoxol test).

Results

1) Differentiation of Yersinia

	SUC	RHAM	RAF	VP (25°C)	IND	LDC	TRE	ODC
Yersinia enterocolitica	$+^0$	0	0	+	V	.	.	.
Yersinia frederiksenii	+	+	0	+	+	.	.	.
Yersinia intermedia	+	+	+	+	+	.	.	.
Yersinia kristensenii	0	0	0	0	V	0	+	+
Yersinia aleksiciae	0	0	0	.	.	+	+	+
Yersinia aldovae	0	+	0	+	0	.	.	.
Other *Yersinia* spp.	V	0	V	0	0	.	.	.

SUC = Sucrose D.T., RHAM = Rhamnose D.T., RAF = Raffinose D.T., VP(25°C) = Voges Proskauer D.T (at 25° C), IND = Indole D.T., TRE = Trehalose D.T., LDC = Lysine Decarboxylase D.T., ODC = Ornithine Decarboxylase D.T.

© Copyright Rosco Diagnostica A/S

2) *Yersinia enterocolitica*

	SUC	PZA	VP
Yersinia enterocolitica (path.)	+	0	+
Yersinia enterocolitica (path. atypical)	0	0	+
Yersinia kristensenii	0	+	0

SUC = Sucrose D.T., PZA = Pyrazinamidase D.T. and VP = Voges Proskauer D.T. All tests performed at **25 °C**.

3) Enterococci resistant to vancomycin (7,8)

	RM	PIGM	ARA	XYL®	FURAZ
Enterococcus faecalis	0	0	0	0	S
Enterococcus faecium	0	0	100	0	R
Enterococcus casseliflavus	96	95	92	·	S
Enterococcus gallinarum	100	0	90	+	S

RM = Rapid motility (incub. 4 h at 35 °C), PIGM = Pigment, ARA = Arabinose D.T., XYL® = Rapid Xylose (incub. 2 h at 37 °C, McF 3) (8), FURAZ = Furazolidone Neo-S.

4) Differentiation of *Streptococcus bovis* biotype I and II

	MAN	NAG
Streptococcus bovis (bio II)	0	0
S. gallolyticus (bovis bio I)	$+^{0}$	$+^{0}$

MAN = Mannitol D.T. and NAG = Beta-N-Acetylglucosaminidase D.T.

5) Differentiation of *Gemella* spp.

	MAL	SUC	SOR	AlkP
Gemella bergeriae	0	0	0	0
Gemella haemolysans	+	V	0	+
Gemella morbillorum	+	+	0^{+}	0
Gemella sanguinis	$+^{0}$	+	+	+

MAL = Maltose D.T., SUC = Sucrose D.T., SOR = Sorbitol D.T. and AlkP = Alkaline Phosphatase D.T.

6) Identification of *Candida glabrata* (9)

	TRE (4h)	SUC (4h)
Candida glabrata	+	0
Candida tropicalis	V	+
Other *Candida* spp.	0	V

TRE = Trehalose D.T., SUC = Sucrose D.T. Incubation 4 h at 37 °C, McF 2.

7) Very rapid (30-60 seconds) identification of *Candida glabrata* (10,11)

	TRE + Clinistix	SUC + Clinistix
Candida glabrata	+	0
Candida tropicalis	V	+
Other *Candida* spp.	0	V

TRE = Trehalose D.T., SUC = Sucrose D.T.
The Diagnostic Tablets are crushed in saline (1 D.T. in 2 ml saline) and the supernatant used for testing.

© Copyright Rosco Diagnostica A/S

8) Differentiation of *Bacillus* spp.

	ANA gr.	NO_3	MAN	LEC
B. subtilis	0	+	+	0
B. cereus	+	$+^0$	0	+
B. megaterium	0	0	+	0

ANA gr. = Anaerobic growth, LEC = Lecithinase.

9) Enterococcus/lactococcus

	SOR	ARA	42 °C
E. faecalis	+	0	+
E. faecium	V	+	+
Lact. garviae	0	0	0

42°C = growth at 42 °C.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Adonitol (Adonitol)	*K. pneumoniae* ATCC 13883	*E. coli* ATCC 25922
l-Arabinose (L-Arabinose)	*E. coli* ATCC 25922	*S. aureus* ATCC 25923
Glucose (D-Glucose monohydrate)	*P. aeruginosa* ATCC 27853	*A. lwoffii* ATCC 9957
Inulin (Inulin)	*S. bovis* ATCC 15351	*E. coli* ATCC 25922
Lactose (Lactose monohydrate)	*E. coli* ATCC 25922	*Proteus vulgaris* ATCC 13315
Maltose (Maltose monohydrate)	*E. coli* ATCC 25922	*Morganella morganii* ATCC 25830
Mannitol (D-Mannitol)	*E. coli* ATCC 25922	*Proteus vulgaris* ATCC 13315
Melibiose (D-Melibiose)	*E. coli* ATCC 25922	*Proteus vulgaris* ATCC 13315
Raffinose (D-Raffinose pentahydrate)	*Enterobacter cloacae* ATCC 13047	*E. coli* ATCC 25922
l-Rhamnose (L-Rhamnose)	*K. pneumoniae* ATCC 13883	*Proteus vulgaris* ATCC 13315
Sorbitol (D-Sorbitol)	*E. coli* ATCC 25922	*Proteus vulgaris* ATCC 13315
Sucrose (Saccharose)	*Enterobacter cloacae* ATCC 13047	*Morganella morganii* ATCC 25830
Trehalose (D-Trehalose dihydrate)	*E. coli* ATCC 25922	*Morganella morganii* ATCC 25830
d-Xylose (d-Xylose)	*E. coli* ATCC 25922	*Morganella morganii* ATCC 25830

References

1) Hollis D.G. et al: Use of the rapid fermentation tests in determining carbohydrate reactions of fastidious bacteria in clinical laboratories. J. Clin. Microbiol. **12**, 620-623, 1980.
2) Farmer III J.J. et al: Pyrazinamidase, CR-MOX agar, Salicin fermentation - Esculin hydrolysis and d-xylose fermentation for identifying pathogenic serotypes of Yersinia enterocolitica. J. Clin. Microbiol. **30**, 2589-94, 1992.
3) Bottone E. Yersinia enterocolitica: the charisma continues. Clin. Microbiol. Reviews, **10**, 257-276, 1997.
4) Guigoule A. et al: Phenotypic and genotypic characterization of virulent Yersinia enterocolitica strains unable to ferment sucrose. J. Clin. Microbiol. **36**, 2732-4,1998.
5) Devriese L.A. et al: Differentiation between Strept. gallolyticus strains of human clinical and veterinary origins and Strept. bovis strains from the intestinal tracts of ruminants. J. Clin. Microbiol. **36**, 3520-3,1998.
6) Collins M. D. et al: Description of Gemella sanguinis sp. nov. isolated from human clinical specimens. J. Clin. Microbiol. **36**, 3090-3, 1998.

© Copyright Rosco Diagnostica A/S

7) Hanson K.L. et al: Comparison of simple and rapid methods for identifying enterococci intrinsically resistant to vancomycin. J. Clin. Microbiol. **37**, 815-7, 1999.
8) Chen D.K. et al: Evaluation of d-xylose and 1% Methyl-α-D-Glucopyramide fermentation tests for distinguishing Ent. gallinarum from Ent. faecium. J. Clin. Microbiol. **38**, 3652-5, 2000.
9) Lopez J. et al. Rapid identification of Cand. glabrata based on trehalose and sucrose assimilation using Rosco Diagnostic Tablets. J. Clin. Microbiol. **39**, 1172-4, 2001.
10) Parant F. et al: A one minute trehalase detection test for Candida glabrata identification. J. Mycol. Med. **11**, 26-31, 2001.
11) Freydiere A.M. et al: Identification of Candida glabrata by a 30 second trehalase test. J. Clin. Microbiol. **40**, 3602-5, 2002.

© Copyright Rosco Diagnostica A/S

3.37 TDA or INDOLE (TDA or IND)

REF No. 57811

Double test tablet that can be used for **either** the Indole test **or** the Tryptophan deaminase test (TDA).

The Tryptophan deaminase test differentiates **Proteus**, **Morganella**, and **Providencia** (positive reaction) from other Enterobacteriaceae (negative reaction) and thus replaces the Phenylalanine deaminase test.

Procedure
Prepare a dense suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a small tube. Add one diagnostic tablet and close the tube. Incubate at 35-37 °C for **4 hours** or **18-24 hours**. The tablet can be used for **either** the Indole test **or** the Tryptophane deaminase test.

Reading of the tests

A) Indole

After incubation add 3 drops of Kovacs' reagent (92031), shake gently and wait for at least 3 minutes. Look only at the **colour of the surface layer**.

Positive reaction: **Red** (purple, pink) (surface layer)
Negative reaction: Yellow

Results

1) The indole test is a well-known test used in the identification of Enterobacteriaceae and in the differentiation of anaerobes.

2) Actinobacillus/Pasteurella

	IND	URE	ONPG
Actinobacillus spp.	0	+	+
Pasteurella spp.	+	0^{+}	0

3a) Differentiation of Propionibacteria (Metro I/R)

	IND	NO_3	ß-XYL	CAT
Propion acnes	+	+	0	+
Propion avidum	0	0	+	+
Propion granulosum	0	0	0	+
Propion propionicum (Arachnia)	0	+	0	0

3b) *Propionibacterium acnes*/Actinomyces

	IND	NO_3	CAT
Prop. acnes	+	+	+
Actinomyces spp.	0	0	0 wk

IND = Indole D.T., URE = Urease D.T., NO_3 = Nitrate Reduction D.T., B-XYL = Beta-Xylosidase D.T., and CAT = Catalase.

© Copyright Rosco Diagnostica A/S

4) Differentiation of Fusobacteria (BrG R, Kana 500 S, Col S, Fosfo S, Vanco 5 R)

	Microscopy	IND	PYR	ESC	Alk P	Rifa	Oxgall	Remarks
Fusobacterium mortiferum	pleomorphic	0	+	$+^0$	+	R (<16 mm)	R	
Fusobacterium necrophorum	pleomorphic	+	0	0	+	S^R	V	subsp. necrophorum LIP + subsp. funduliformis LIP 0
Fusobacterium nucleatum	fusiform (slender)	+	0^+	0	0	S (≥16 mm)	S	(zone) NO_3 0, PGUA 0
Fusobacterium varium		$+^0$	+	0	V	R	R	(no zone)
Fusobacterium gonidiaformans	non fusiform	+	.	0	0	S	S	HIP +
Fusobacterium naviforme	boat shape	+	.	0	0	S	S	PGUA +

IND = Indole D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., ESC = Esculin Hydrolysis D.T., Alk P = Alkaline Phosphatase D.T., Rifa = Rifampicin Neo-S, Oxgall (S = zone, R = no zone). BrG = Brilliant Green D.T. (S ≥ 10 mm, R < 10 mm), Kana 500 = Kanamycin 500 µg (S ≥ 10 mm, R < 10 mm), Col = Colistin 10 Neo-S (S ≥ 10 mm, R < 10 mm), Fosfo = Fosfomycin Neo-S (S ≥ 20 mm), Vanco 5 = Vancomycin 5 µg Neo-S (S ≥ 20 mm, R ≤ 18 mm).

B) <u>Tryptophane deaminase (TDA)</u>

After incubation add 2 drops of Ferric Chloride 10% solution. Read within 5 minutes.

Positive reaction: **Brown/red**
Negative reaction: Yellow/orange

Indole positive strains may produce an orange colour due to indole production. This is a negative reaction.

Results

	TDA
Proteus spp.	+
Morganella morganii	+
Providencia spp.	+
Other Enterobacteriaceae	0

Quality Control

DIATABS (Active ingredients)	Positive	Negative
TDA or Indole: Indole (L-Tryptophane)	*Proteus vulgaris* ATCC 13315	*K. pneumoniae* ATCC 13883
TDA or Indole: TDA (L-Tryptophane)	*Proteus vulgaris* ATCC 13315	*K. pneumoniae* ATCC 13883

References

1) Funke G. et al: Clinical microbiology of Coryneform bacteria. Clin. Microbiol. Reviews **10**, 125-159, 1997.
2) Ashhurst-Smith C. et al: Actinobacillus equuli septicemia: an unusual zoonotic infection. J. Clin. Microbiol., **36**, 2789-90, 1998.

© Copyright Rosco Diagnostica A/S

3.38 TELLUR (TEL)

REF No. 45011

Contain potassium tellurite and are specially intended for the differentiation of ***Enterococcus faecalis*** from other enterococci and **streptococci**. The diffusible amount is 500 µg per tablet.

Procedure
Place one Tellur diagnostic tablet on an agar plate seeded with the culture to be tested and incubate overnight at 35-37 °C.

Enterococcus faecalis will normally grow on a tellurite containing substrate with black colonies, i.e. it will grow close to the edge of the Tellur diagnostic tablet showing a broad ring of black colonies, whereas other enterococci and streptococci will grow relatively far from the tablet.

Reading of the test

		TEL
E. faecalis:	**Grey/black colonies**, close to the edge of the tablet.	< 12 mm (R)
Streptococci and most other enterococci:		> 15 mm (S)

A few Tellur resistant strains are found among *E. casseliflavus*, *E. mundtii*, *E. faecium*, and *E. gallinarum*.

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
Tellur 500 µg (Potassium tellurite)	*S. bovis* ATCC 15351	*E. faecalis* ATCC 29212

Reference
1) Facklam R.R.: Recognition of group D streptococcal species of human origin by biochemical and physiological tests. Appl. Microbiol. **23**, 1131-1139, 1972.

© Copyright Rosco Diagnostica A/S

3.39 TETRATHIONATE REDUCTASE (TTR)

REF No. 57421

The enzyme tetrathionate reductase catalyzes the reduction of tetrathionate into thiosulphate. It has a diagnostic interest in the case of gram negative facultative anaerobes (Enterobacteriaceae, Vibrionaceae, etc.) and also in the case of gram negative strictly aerobes (Non-fermenting gram negative bacilli).

Procedure
Prepare a dense "milky" suspension (at least McFarland No. 4) from the strain to be tested in 0.25 ml saline in a tube. Add one TTR Diagnostic Tablet and **3 drops of sterile paraffin oil**. Close the tube and incubate at 35-37 °C for **4 hours** or for 18-24 hours.

Reading of the test
Positive reaction: **Yellow**
Negative reaction: Red/orange

The oil overlay provides anaerobic conditions necessary to avoid false positive reactions.

Results

1) Enterobacteriaceae

TTR positive	TTR negative
Edwardsiella	*E. coli*
Salmonella $+^0$	*Shigella* spp.
Citrobacter freundii	*Klebsiella* spp.
Serratia liquefaciens	*Enterobacter* spp.
Proteus spp.	*Serratia marcescens* (V)
Morganella	*Yersinia enterocolitica* (V)
Providencia spp.	

2) Non-fermenters

TTR positive	TTR negative
Comamonas acidovorans	*Ps. fluorescens*
Com testosteroni	*Ps. putida*
Shewanella putrefaciens	*Sphing. paucimobilis*
Shewanella algae	*Burkh. cepacia*
Sten. maltophilia	*Brev. diminuta*
Alcalig denitrificans $+^0$	*Ralst. pickettii*
Achr. xylosoxidans	*Acinetobacter* spp.
Past. multocida	

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Tetrathionate reductase (Tetrathionate)	*Proteus vulgaris* ATCC 13315	*E. coli* ATCC 25922

Reference
1) Richard C.: La tetrathionate-reductase (TTR) chez les bacilles a gram negatif: Interet diagnostique et epidemiologique. Bull. Inst. Pasteur, **75**, 369-382, 1977.
2) Freland C. et al: Campylobacter pyloridis: etude bacteriologique et sensibilite aux antibiotiques. Path. Biologie **35**, 1037-1042, 1987.
3) Le Minor L: Tetrathionate reductase, beta glucuronidase and ONPG-test in the genus Salmonella. Zentralbl. Bakteriol. (Orig A), **243**, 321-5, 1979.

© Copyright Rosco Diagnostica A/S

3.40 TRIBUTYRIN (TRIB)

REF No. 48821

Test for enzymatic hydrolysis of tributyrin into butyric acid and glycerol. The release of butyric acid lowers pH and results in a colour change from red to yellow. Mainly used in **differentiation of *Moraxella catarrhalis*** (positive within 4 hours) from *Neisseria* spp. (negative).

Procedure
Growth from an agar plate (oxidase positive, gram-negative diplococci) is suspended in 0.25 ml saline to achieve a turbidity corresponding to McFarland No. 4-5. Add one Tributyrin diagnostic tablet and close the tube. Incubate at 35-37 °C for **4 hours**. It is also possible to read after overnight incubation.

Reading of the test
Positive reaction: **Yellow**, yellow orange
Negative reaction: Red

Results

1) Moraxella/Neisseria

	TRIB
Moraxella catarrhalis	+
Neisseria spp.	0

2) Coryneform bacteria

	TRIB	PGUA
Coryn. glucuronolyticum	+	+
Coryn. renale	0	0

PGUA = Beta-Glucuronidase.

3) Differentiation of Moraxella/Psychrobacter

	TRIB	GLU	42 °C	URE	NO_3	Alk P	Remarks
M. catarrhalis	+	.	0	0	$+^0$	+	
M. nonliquefaciens	0	.	+	0	+	0	PRO +
M. lacunata	0	.	0	0	+	+	
M. osloensis	0	.	+	0	V	+	Yellow pigm., PYR 0
M. atlantae	0	.	0	0	0	+	PYR +, McConk + Growth stim. bile
Ps. phenylpyruvicus	0	+	0	+	V	+	Growth stim. bile
Ps. immobilis	$+^0$	+	0	+	+	V	DEFRX S, growth not stim. bile
Ps. phenylpyruvicus-like	+	0	0	+	+	.	Growth stim. bile

GLU = Glucose D.T., 42 °C = growth at 42 °C, URE = Urease D.T., NO_3 = Nitrate Reduction D.T., Alk P = Alkaline Fosfatase D.T., PRO = Proline Aminopeptidase D.T., McConk. = Growth in McConkey, Growth stim. bile = Growth stimulated by bile, DEFRX = Deferoxamine D.T. (S ≥ 16 mm, R ≤ 14 mm).

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Tributyrin (Tributyrin)	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922

© Copyright Rosco Diagnostica A/S

References

1) Riou J.Y. et al: Hydrolyse de la tributyrine par les Neisseria et les Branhamella. (French). Ann.Microbiol. (Inst. Pasteur), **132A**, 159-169, 1981.
2) Riou J.Y., Guibourdenche: Branhamella catarrhalis. New methods of bacterial diagnosis. Drugs (suppl. 3), 1-6, 1986.
3) Christensen J.J. et al.: Branhamella catarrhalis: significance in pulmonary infections and bacteriological features. Acta path. microbiol. immunol scand, Section B, **94**, 89-95, 1986.
4) Richards J.: Evaluation of a rapid method for identifying Branhamella catarrhalis. J. Clin. Pathol. **41** ,462-464, 1988.
5) Cooke R.P.D.: Laboratory diagnosis of Branhamella catarrhalis. J. Clin. Pathol. **41**, 923, 1988.
6) Mannion P.T.: Tributyrin hydrolysis for identifying Branhamella catarrhalis. J. Clin. Pathol. **42**, 115, 1989.
7) Perez J.L. et al: Butyrate esterase (Tributyrin) spot test, a simple method for immediate identification of Moraxella catarrhalis. J. Clin. Microbiol. **28**, 2347-8, 1990.
8) Früh M. et al: Use of second line biochemical and susceptibility tests for the differential identification of coryneform bacteria. Clin. Microbiol. Infect. **4**, 332-8, 1998.
9) Verduin C.M. et al: Moraxella catarrhalis: from emerging to established pathogen. Clin. Microbiol. Reviews **15**, 125-144, 2002.

© Copyright Rosco Diagnostica A/S

3.41 UREASE (URE)

REF No. 57511

The hydrolysis of urea is catalyzed by a specific enzyme, urease, to yield two molecules of ammonia. In the presence of the indicator phenol red there is a change of colour from yellow/orange to red/ purple in case of a positive reaction.

Procedure
Prepare a dense "milky" suspension (at least McFarland No. 4) from the strain to be tested in 0.25 ml saline in a tube. Add one Urease diagnostic tablet, close the tube and incubate at 35-37 °C for **4 hours** or **18-24 hours**.

Reading of the test
Positive reaction: **Red/purple**
Negative reaction: Yellow/orange

After **overnight** incubation **only strong red or purple** should be considered as a positive reaction!

Results

1) **Enterobacteriaceae**
The rapidity by which there is a change of colour (urease pos.) may have a diagnostic interest.

Morg. morganii show in most cases a **positive reaction** within 30 min.

The following species of Enterobacteriaceae usually show a **positive reaction** within 4 hours: *Proteus* spp., *Morg. morganii*, *Enterobact. gergoviae*.

Klebsiella pneumoniae/*Klebs. oxytoca* and *Yersinia* spp. show also **a positive reaction**, but in most cases after overnight incubation.

The remaining Enterobacteriaceae show a **negative reaction**.

2) **Staphylococci**
The following staphylococci usually show a **positive reaction**:
Staph. epidermidis, *Staph. hominis*, *Staph. warneri*, *Staph. simulans*, *Staph. saprophyticus*, *Staph. xylosus*, *Staph. aureus* (V), *S. lugdunensis* (V), *Staph. capitis* subsp. *ureolyticus*, *Staph. cohnii* subsp. *urealyticum*.

The following staphylococci show a **negative reaction**:
Staph. capitis, *Staph. haemolyticus*, *Staph. auricularis*, *Staph. schleiferi*, *Staph. cohnii*, *Staph. sciuri*, *Staph. lentus*.

3) **Non-fermenters**
The following non-fermenters usually show a **positive reaction**:
Flav. odoratum, *Ochrobactrum anthropi* (Group Vd), *Sph. multivorum*, CDC group IV c-2, *Oligella ureolytica* (IVe), *Bordetella bronchiseptica*, *Agrob. tumefaciens* (radiobacter).

4) **Differentiation of Actinobacillus from Pasteurella**

	URE	IND	ONPG
Actinobacillus spp.	+	0	+
Pasteurella spp.	0^{+}	+	0

URE = Urease D.T., IND = Indole D.T.

5) **Useful in identification of gram-negative anaerobes:**
Bact. ureolyticus (+), *Bilophila* spp. (+0), *Desulfomonas pigra* (V). Others are negative.

© Copyright Rosco Diagnostica A/S

6) Differentiation of <u>lipophilic</u> corynebacteria

Most strains are: CAT +, PRO $+^0$, Fosfo R, Mupi R, MOT 0, Col R, Nali R.

	URE	NO_3	PZA	HIP	GLU	SUC	AlkP	Remarks
C. accolens	0	+	V	·	+	V	0	αGLU +
C. afermentans ssp. *lipophilicum*	0	0	+	0	0	0	$+^0$	LAP +
C. bovis	0^+	0	V	$+^0$	+	0	+	ONPG +
CDC group F-1 *C. pseudogenitalium*	+	V	+	0	+	+	0	
CDC group G	0	V	+	V	+	V	+	FRU +
C. jeikeium (JK)	0	0	+	+	+	0	+	αGLU 0, Res., FRU 0
C. macgingleyi	0	+	0	V	+	+	+	LAP 0
C. urealyticum (D-2)	+	0	+	$+^0$	0	0	V	O/129 R^S
C. resistens	0	0	0	·	+	0	+	PYR +, α-GLU 0, Res.

URE = Urease D.T., NO_3 = Nitrate Reduction D.T., HIP = Hippurate Hydrolysis D.T., GLU = Glucose D.T., SUC = Sucrose D.T., AlkP = Alkaline Phosphatase D.T., αGLU = Alpha-Glucosidase S.D., LAP = Leucine Aminopeptidase D.T., PZA = Pyrazinamidase D.T., O/129 = O/129 D.T. (S ≥ 16 mm, R < 16 mm), Res = multiresistant, CAT = catalase, PRO = Proline Aminopeptidase, Fosfo = Fosfomycin Neo-S (R = no zone), Mupi = Mupirocin Neo-S (R = no zone), MOT = motility, PZA = Pyrazinamindase D.T., FRU = Fructose D.T..

7) Differentiation of *Brucella* spp. from similar organisms

All inoculation procedures and manipulation of possible cultures of *Brucella* spp. should be performed wearing gloves, in a biological safety cabinet.

	CAT	OXI	URE	NO_3	MOT	Cell morphol.	Remarks
Brucella spp.	+	+	$+^R$	+	0	Tiny cbb stains faint	from blood, bone marrow, X or V factor not req.
Bordetella bronchispetica	·	+	+	+	+	small ccb, rods	
Acinetobacter spp.	·	0	V	0	0	broad ccb	
Psychrobacter spp.	·	+	+	V	0	broad ccb	
Oligella ureolytica	·	+	$+^R$	+	V	tiny ccb	from urine
Pasteurella spp.	·	+wk	0^+	+	0	medium rods	IND +
Francisella tularensis	·	0	0	0	0	tiny ccb	
Haemophilus influenzae	·	V	V	+	0	small ccb	X or V factor req.

OXI = Oxidase D.T., URE= Urease D.T., NO_3 = Nitrate Reduction D.T., MOT = motility, $+^R$ = rapid positive reaction, ccb = coccobacilli, IND = Indole D.T., wk = weak.

1) *Brucella* spp. appear as punctate smooth colonies, non pigmented, non haemolytic.
2) *Brucella* spp. do not grow on McConckey Agar.
3) *Brucella* spp. have a characteristic Gram stain morphology: tiny, faintly stained coccobacilli (differentiation from *Psychrobacter* spp.).
4) *Brucella* spp. are oxidase and urease positive (in most cases rapidly positive), and X or V Factors are not required for growth (differentiation from *haemophilus* spp.).
5) The most common misidentification of *Brucella* spp. is *Haemophilus influenzae* (requires X and V Factors) and *Psychrobacter* spp. (broad coccobacilli).

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Urease (Urea)	*Proteus vulgaris* ATCC 13315	*E. coli* ATCC 25922

© Copyright Rosco Diagnostica A/S

References

1) Jousimies-Somer H.R. et al: Anaerobic gram-negative bacilli and cocci. Manual of Clinical Microbiology 5th Ed., 538-552, 1991.
2) Summanen P. et al: Wadsworth anaerobic Bacteriology Manual 5th Ed. Advanced Identification Methods (Level III) pages (49, 50, 65, 93, 159) 1993.
3) Ashhurst-Smith C. et al: Actinobacillus equuli septicemia: an unusual zoonotic infection. J. Clin. Microbiol. **36**, 2789-90, 1998.

© Copyright Rosco Diagnostica A/S

3.42 VOGES-PROSKAUER (VP)

REF No. 57711

The Voges-Proskauer test is used in the differentiation among **Enterobacteriaceae** and in the **streptococci** group.

Procedure
Prepare a dense bacterial suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one Voges-Proskauer Diagnostic Tablet and close the tube. Incubate at 35-37 °C for not more than **4 hours**.

Before reading, **add 2 drops of alpha-naphthol solution** (5% in ethanol) and afterwards 1 drop of 40% KOH and shake.

Reading of the test
Positive reaction: **Red/pink**
Negative reaction: Colourless (no change in colour) . Very light pink.

Wait 5 min before a test is considered negative.

Results

1) Enterobacteriaceae

	VP
Enterobacter spp., *Klebsiella pneumoniae*/*oxytoca*, *Serratia* spp., Hafnia	positive
Citrobacter spp., *E. coli*, *Klebsiella ozaenae*/*rhino-scleromatis*, *Morganella morganii*, *Proteus* spp. (except *Pr. mirabilis*: V), *Providencia* spp., *Salmonella* spp., *Shigella* spp.	negative

2) Yersinia

	VP(25°C)	SUC	PZA
Yersinia enterocolitica	+	$+^{0}$	0
Yersinia kristensenii	0	0	+

VP(25°C) = Voges Proskauer D.T. (at 25 °C), SUC = Sucrose D.T. and PZA = Pyrazinamidase D.T.

3a) Streptococci beta-haemolytic (human) (8) (Mupi S)

	VP(4h)	PYR	PGUA	HIP	CAMP	Colony Size	Remarks
S. pyogenes (Group A)	0	+	V	0	0	Large	SORB 0, MAN 0
S. anginosus (Groups A, C, G, F)	+	0	0	0	0	Small	
Group B (*S. agalactiae*)	+	0	$+^{0}$	+	+	.	SORB 0, MAN 0
S .dysgalactiae subsp. *equisimilis* (ACG) *	0	0	+	0^{w}	0	Large	αGAL 0, TRE +, SORB 0
S. iniae (no group)	0	+	+	0	+		SORB 0, MAN +
S. porcinus (E, P, U, V)	+	$+^{0}$	+	0^{+}	+		SORB +, MAN +
S. pseudoporcinus	V	0^{+}	.	0	.	Small	SORB +, MAN +, ADH +, ESC +, LAP +
S. canis (G)	0	V	0	0	+	Large	SORB 0, αGAL +, MAN 0
S. equi subsp. *zooepidemicus* (C)	0	0	+	0	0	Large	SORB +, TRE 0
Arcanobact. haemolyticum	0	0	V	0	.		Mupi R

* may be alpha haemolytic sometimes.

© Copyright Rosco Diagnostica A/S

VP(4h) = Voges Proskauer D.T. (4 h incubation), PYR = Pyrrolidonyl Aminopeptidase D.T., PGUA = Beta-Glucuronidase D.T., HIP = Hippurate Hydrolysis D.T., Mupi = Mupirocin Neo-S (S ≥ 16 mm, R < 16 mm), CAMP = CAMP test.

3b) Viridans streptococci (most current clinical isolates)

Most strains are: CAT 0, LAP +, PYR 0, BE 0.

A1) *S. mitis* group (VP 0)

	OPT	ADH	ONPG	α-GAL	α-FUC	TRE	ESC	Remarks
S. gordonii	R	+	+	V	+	+	+	PGUA 0, ß-MAN +, SUC +
S. parasanguinis	R	+	+	$+^0$	V	0^+	0	
S. sanguinis	R	+	0^+	V	0	+	+	AlkP 0, SUC +
S. mitis	R/S	0^+	$+^0$	+	0	0	0	(Oxgall R), AMYG +
S. oralis	R	0	$+^0$	0	0	V	0	Oxgall R, AMYG 0
S. suis	R	+	+	+	+	+	+	PGUA $+^0$, CAMP +, PYR $+^0$, MAN 0
S. gallinaceus	R	+	+	+	+	+	+	PGUA 0, MAN +
S. massiliensis	R	+	0	0	0	0	0	HIP +, LAP +, AlkP +, MAL +, SUC 0
S. pneumoniae	S	$+^0$	+	+	0	+	V	Oxgall S
S. pseudopneumoniae	R/S	+	+	0	.	V	V	Oxgall R, TET R^s, ERY R^S

A2) *S. sanguinis* biotypes

	ESC	α-GAL	ONPG
S. sanguinis bio 1	+	V	0
S. sanguinis bio 2	+	+	V
S. sanguinis bio 3	0	0	+

B) *S. "milleri"*/anginosus group (ADH +, ESC +, MAN 0, PYR 0, VP +, SOR 0)

	NAG	ONPG	RAF	ß-FUC	ß-GLU
S. anginosus	0	0	V	0	+
S. constellatus	0	0	0	0	0
S. constellatus subsp. *pharyngis*	+	+	0	+	$+^0$
S. intermedius	+	+	0^+	+	V

C) *S. mutans* group (ADH 0, VP +, MAN +)

	RAF	BaL	ß-GLU
S. mutans	$+^0$	R	+
S. sobrinus	0	R	0
S. cricetus	+	S (≥10 mm)	
S. downei	0	S	

D) *Bovis* group (ADH 0, most VP + and MAN 0)

	ONPG	α-GAL	ESC	PGUA	TRE	MAN	Remarks
S. infantarius	0	+	0^+	0	0	0	MEL +
S. lutetiensis	0	$+^0$	+	0	0	0	MEL 0
S. gallolyticus (*bovis* I)	0	V	+	0	+	+	BE +
S. macedonicus	$+^0$	0^+	0	0	0	0	
S. pasteurianus	+	V	+	+	+	0	ß-MAN +
S. bovis (II)	0	$+^0$	+	0	V	0	BE +,VP 0

ADH = Arginine Dihydrolase D.T., α-GLU = Alpha-Glucosidase D.T., α-GAL = Alpha-Galactosidase D.T., α-FUC = Alpha-Fucosidase D.T., ESC = Esculin Hydrolysis D.T., NAG = Beta-N-Acetylglucosaminidase D.T., ß-FUC = Beta-

© Copyright Rosco Diagnostica A/S

Fucosidase D.T., ß-GLU = Beta-Glucosidase D.T., RAF = Raffinose D.T., BaL = Bacitracin Low D.T., VP = Voges Proskauer D.T. (4 hours incubation), Col. dry adh. = Colonies dry adherent. OPT = Optochin 10 µg D.T., TRE = Trehalose D.T., AMYG = Amygdalin.

4a) Coagulase positive staphylococci

	VP(4h)	PYR(1h)	Poly	PGUA
Staphylococcus aureus	+	0 wk	R (≤ 12 mm)	0
S. intermedius	0 wk	+	S (≥ 14 mm)	0
S. pseudintermedius	+	+	S (≥ 14 mm)	0
S. hyicus	0	0 wk	V	+
S. schleiferi coagulans	+	+	S (≥ 14 mm)	0

VP(4h) = Voges Proskauer D.T. (4 h. incubation), PYR = Pyrrolidonyl Aminopeptidase D.T. (1 h. incubation), Poly = Polymyxins Neo-S, PGUA = Beta-Glucuronidase D.T.

4b) Differentiation of gram positive cocci from blood cultures (most common)

	PYR (1h)	αGAL	HIP	VP	Remarks
S. aureus	0	·	·	+	HCF +
St. pneumoniae	0	+	0	0	OPT S
Enterococci		V	V	+	BE+, GrD
St. group A		0^{+}	0	0	
St. group B	0	0	+	+	HCF 0

αGAL = Alpha-Galactosidase D.T., HIP Hippurate Hydrolysis D.T., VP = Voges Proskauer D.T., PYR = Pyrrolidonyl Aminopeptidase (1 hour incubation), HCF = Human clumping factor, OPT = Optochin D.T. (S ≥ 18 mm, R < 16 mm), BE = Bile Esculin D.T., GrD = Group D.

5) *Arcanobacterium* spp.

	VP(24h)	α-MAN	PYR	TRIB	XYL
Arcanob. pyogenes	+	0	82	0	+
Arcanob. haemolyticum	0	+	0	70	0

VP(24h) = Voges Proskauer D.T. (24h incubation), α-MAN = Alpha-Mannosidase D.T., PYR = Pyrrolidonyl Amino-peptidase D.T., TRIB = Tributyrin D.T., XYL = Xylose D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Voges-Proskauer (Sodium Pyruvate 2 mg, Creatine)	*Enterobacter cloacae* ATCC 13047	*E. coli* ATCC 25922

References

1) Devriese L.A. et al: Streptococcus hyointestinalis sp. nov. from the gut of swine. Intl. J. Syst. Bacteriol **38**, 440-1, 1988.
2) Devriese L.A. et al: Identification of Enterococcus spp. isolated from foods of animal origin. Intl. J. Food Microbiol. **26**, 187-197, 1995.
3) Mahoudeau I. et al: Frequency of isolation of Staph. intermedius from humans. J. Clin. Microbiol. **35**, 2153-4, 1997.
4) Guiyoule A. et al: Phenotypic and genotypic characterization of virulent Yersinia enterocolitica strains unable to ferment sucrose. J. Clin. Microbiol. **36**, 2732-4, 1998.
5) Carlson P. et al: Additional tests to differentiate Arcanobacterium haemolyticum and Actinomyces pyogenes. Zentralbl. Bakteriol. in press.
6) Claridge III J.E. et al: Genotypic and phenotypic characterization of "Streptococcus milleri" group isolates from a Veteran Administration Hospital population. J. Clin. Microbiol. **37**, 3681-87, 1999.
7) Ruoff K. et al: Streptococcus in Manual of Clinical Microbiology. 7th ed. **17**, 283-296, 1999.
8) Brandt C.M. et al: Characterization of blood culture isolates of Str. dysgalactiae subsp. equisimilis possessing Lancefield's group A antigen. J. Clin. Microbiol. **37**, 4194-7, 1999.

© Copyright Rosco Diagnostica A/S

4 Reagents

Reagents are used together with some of the Diatabs. An overview of these Diatabs is given in the table. Ninhydrin Solvent, Aminopeptidase and Kovacs' Reagents are available from Rosco. The other reagents are easily prepared. Follow the safety guidelines for the chemicals being used. For quality control use the reagent together with the recommended Diatabs when testing positive and negative QC strains.

Reagent	REF No.	Use with Diatabs (REF No.)
Aminopeptidase Reagent	92231	46711, 46811, 46911, 47011, 47211, 58011
Kovacs' Reagent	92031	57611, 58411, 59121, 59011, 57611 (IND)
Ninhydrin Solvent	91731	56711 (HIP)
N,N-Dimethyl-α-Naphtylamine		43711 (NO_3)
Sulfanilic acid solution		43711 (NO_3)
Ferric Chloride 10 % solution		57911, 57811 (TDA)
Alpha-naphthol solution		57711 (VP)
40 % KOH		57711 (VP)
Ferrous ammonium sulphate solution		59811 (PZA)

N,N-Dimethyl-α-Naphthylamine:
Dissolve 600 mg N,N-Dimethyl-α-Naphthylamine (Sigma D 4011 or Fluka 40860) in 30 ml Acetic acid 100 % and dilute to 100 ml with water, purified. Store in the refrigerator in a brown glass bottle away from light.

Sulfanilic acid solution:
Dissolve 800 mg Sulfanilic acid i 30 ml Acetic acid 100% and dilute to 100 ml with water, purified. Store in the refrigerator in a brown glass bottle away from light.

Ferric Chloride 10 % solution:
Dissolve 10 g ferric chloride $FeCl_3 \cdot 6\ H_2O$ in water, purified to make 100 ml.

Alpha-naphthol solution:
Dissolve 5 g α-naphthol in 100 ml of absolute ethanol. Store in the refrigerator in a brown glass bottle away from light.

40 % KOH:
Dissolve 40 g of potassium hydroxide in 100 ml of carbon dioxide free water, purified.

Ferrous ammonium sulphate solution 5%:
Dissolve 5 g of ferrous ammonium sulfate in 100 ml of purified water. Use only freshly prepared or stored at –20 °C.

© Copyright Rosco Diagnostica A/S

5 Useful TABLES for bacterial identification / differentiation

1) Enterobacteriaceae
2) Non-Fermenters
3) Vibrio / Aeromonas / Plesiomonas
4) Staphylococci / Micrococci / Kitococcus
5) Enterococci
6) Streptococci / Pneumococci
7) Catalase Negative, Gram Positive Cocci
8) Pediococcus / Leuconostoc / Enterococcus
9) Arcanobacterium
10) Neisseria / Moraxella / Psychrobacter / Brucella
11) Haemophilus / HACEK Group / Aggregatibacter
12) Corynebacteria
13) Gardnerella / Mobiluncus
14) Actinobacillus / Pasteurella
15) Actinomyces
16) Campylobacter / Helicobacter
17) Bacillus
18) Anaerobes
19) Nocardia
20) Yeast

Pages

1) ENTEROBACTERIACEAE

Identification of *E. coli* (PGUA/Indole) 50
Identification of Salmonella / Shigella (LOUIS test) 47
Differentiation of Enterobacteriaceae 21, 77
Differentiation of Klebsiella/ Enterobacter / Serratia 77
Differentiation of H_2S positive (TTR +) members of Enterobacteriaceae 21
Differentiation of Salmonella / Citrobacter 20
Differentiation of *Citrobacter* spp. 48
Differentiation of Proteus / Morganella / Providencia from others 53, 112
Differentiation of Enterobacter 29
Differentiation of *Enterobacter sakazakii* from other *Enterobacter* spp. 73
Differentiation of *Yersinia* spp. 107, 120
Differentiation of *Yersinia enterocolitica* pathogenic serotype 38, 103, 108
Salmonella and Shigella serotypes 14

2) NON-FERMENTERS

Differentiation of most important non-fermenters 23
Differentiation of most common resistant non-fermenters 21
Identification of *Pseudomonas aeruginosa* (C-390, Ps.aeruginosa Screen) 41, 102
Differentiation of *Ps. fluorescens, Ps. putida* and *Ps. stutzeri* 25
Differentiation of *Acinetobacter baumanii / lwoffi* 13, 77
Differentiation of *Burkholderia cepacia* complex 25
Differentiation of *Ralstonia / Cupriavidus* spp. 45
Differentiation of *Shewanella alga / Shewanella putrefaciens* 97
Differentiation of gram negatives from cystic fibrosis patients 102
Differentiation between *Burkholderia*, *Ralstonia* and *Pandoraea* spp. 22
Differentiation of *Burkholderia cepacia* complex, *B. gladioli*, *R. picketti* and *R. manitolilytica* 25

© Copyright Rosco Diagnostica A/S

Pages

3) VIBRIO / AEROMONAS / PLESIOMONAS

Differentiation of Vibrio, Aeromonas, Plesiomonas, Photobacterium 92
Differentiation of *Aeromonas* spp. 92
Differentiation of the most common *Vibrio* spp. (human) 49
Differentiation of Vibrio/Enterobacteriaceae/Pasteurellaceae 92

4) STAPHYLOCOCCI / MICROCOCCI / KITOCOCCUS

Identification of most important staphylococci 10
Identification of most common human staphylocci 20
Differentiation of coagulase negative staphylococci (human) 44, 58
Differentiation of coagulase negative mastitis staphylococci 45, 58
Differentiation of coagulase positive staphylococci 20, 45, 122
Differentiation of staphylococci from micrococci and kitococcus 60
Differentiation of *S. aureus / S. intermedius / S. pseudintermedius* 20
Differentiation of *S. haemolyticus / S. hominis / S. lugdunensis* 20, 84
Identification of *S. lugdunensis* (ODC +, PYR +) 84
Differentiation of *S. saprophyticus* group 90
Staph. hominis / *S. epidermidis* 90

5) ENTEROCOCCI

Differentiation of enterococci 60, 113
Differentiation of enterococci resistant to vancomycin 108
Differentiation of enterococci from *Lactococcus garviae* 109

6) STREPTOCOCCI / PNEUMOCOCCI

Differentiation of beta-haemolytic streptococci (human) 120
Identification of *S. pyogenes* 20, 31
Identification of group B streptococci 79
Differentiation of the "milleri" anginosus group 64, 69, 121
Identification of gram positives from throat cultures 31, 61
Differentiation of *viridans streptococci* 121
Differentiation of *S. gordonii* from *S. sanguinis* 121
Differentiation of *S. bovis* from *S. gallolyticus* 39, 108
Differentiation of *S. bovis* I/II, *S. mutans* and *E. faecalis* 39
Identification of pneumococci 94
Differentiation of *S. pneumoniae*, *S. pseudopneumoniae* and *S. mitis/oralis* group 94
Differentiation of gram positive cocci from blood cultures 122
Streptococci from subclinical mastitis 79
Differentiation of Group C and G beta-haemolytic streptococci 69

7) CATALASE NEGATIVE, GRAM POSITIVE COCCI

Differentiation of the different genus 16
Catalase negative cocci from milk 80
Differentiation of *Aerococcus* spp. 17
Differentiation of *Abiotrophia* and *Granulicatella* spp. 30, 70, 80
Differentiation of *Gemella* spp. 10, 108
Differentiation of *Facklamia* spp. 80
Differentiation of Globicatella and Aerococcus 17
Phenotypic patterns of *Aerococcus urinae* 73

8) PEDIOCOCCUS / LEUCONOSTOC / ENTEROCOCCUS

Differentiation of vancomycin resistant lactococci / coccobacilli (human) 21, 38
Differentiation of *Pediococcus* spp. 91

© Copyright Rosco Diagnostica A/S

Pages

9) ARCANOBACTERIUM

Arcanobacterium haemolyticum biotypes 75
Differentiation of *Arcanobacterium pyogenes/A. haemolyticum* from *Dermabacter hominis*/Listeria . 21, 76, 122
Throat cultures (Arcanobacterium/streptococci) 31, 61

10) NEISSERIA / MORAXELLA / PSYCHROBACTER / BRUCELLA

Identification / differentiation of *Moraxella catarrhalis* 115
Differentiation of *Neisseria* spp. / Moraxella 13
Differentiation of cocco-bacillary *Neisseria* spp. / *Kingella* spp. / Moraxella / Psychrobacter / Pasteurella 88, 97
Differentiation of *Moraxella* spp. / Psychrobacter 115
Differentiation of *Brucella* spp. from similar organisms 118

11) HAEMOPHILUS / HACEK GROUP /AGGREGATIBACTER

Identification of Haemophilus (Factor X, V, X+V, ALA) 56, 99
Screening of Haemophilus in throat/sputum cultures 32
Differentiation of the HACEK group of microorganisms (+ Capnocytophaga) 71
Differentiation of biotypes of *Haemophilus influenzae* 49
Differentiation of *Aggregatibacter* spp. 100

12) CORYNEBACTERIA

Differentiation of lipophilic corynebacteria 118
Differentiation of nonlipophilic – fermentative spp. 16, 93
Differentiation of of nonlipophilic – nonfermentative spp. 17
Differentiation of *C. diphtheriae* from *C. imitans* and *C. striatum* 93, 103
Differentiation of *C. minutissimum* from *C. amycolatum*, *C. striatum*, *C. xerosis* 61, 93
Differentiation of *C. glucuronolyticum* from *C. renale* 115
LDC +, ODC + coryneforms 84
Differentiation of Corynebacterium from *Listeria* spp. 76

13) GARDNERELLA / MOBILUNCUS

Identification of *Gardnerella vaginalis* 31, 73, 80, 87, 105
Differentiation of *Mobiluncus* spp. 80

14) ACTINOBACILLUS / PASTEURELLA

Differentiation of *Pasteurella* spp. (human interest) 51
Differentiation of *Actinobacillus* spp. from *Pasteurella* spp. 51, 71, 111
Differentiation of *Actinobacillus* spp. 71
Differentiation of Vibrio/Enterobacteriaceae/Pasteurellaceae 92

15) ACTINOMYCES

Identification of Actinomyces and related species 39
Differentiation of *Actinomyces europaeus* / *A. radingae* / *A. turicensis* 64, 71
Differentiation of *Actinomyces gerencseriae / A. israelii* 76
Differentiation of *Actinomyces* spp. from *Propionibacterium acnes* 111

16) CAMPYLOBACTER / HELICOBACTER

Differentiation of *Helicobacter* spp. isolated from human blood 82
Differentiation of *H. pylori* / *H. cinaedi* / *H. fennelliae* 13
Differentiation of *Campylobacter jejuni* 79
Differentiation of enteropathogenic Campylobacters / *Arcobacter butzleri* 80
Differentiation of *C. curvus*, *C. jejuni*, *W. succinogenes* and *H. pylori* 83
Differentiation of emerging *Campylobacter* spp., Arcobacter and Helicobacter from stools 83

© Copyright Rosco Diagnostica A/S

Pages

17) BACILLUS

Differentiation of *B. subtilis* / *B. cereus* / *B. megaterium* 109

18) ANAEROBES

Presumptive identification of anaerobes (Oxgall, Brilliant Green, Vanco 5, Kana 500, COL 10 µg) .. 27
Screening of gram-negative anaerobes (*B. fragilis*, Prevotella, Porphyromonas, Fusobacteria) 27
Differentiation of *Bacteroides fragilis* group 67
Differentiation of Bacteroides / Parabacteroides 68
Differentiation within *Parabacteroides* spp. 68
Differentiation of *Fusobacterium* spp. 112
Differentiation of *Porphyromonas* spp. 23, 66
Differentiation of *Prevotella* spp. 66
Differentiation of pigmented gram-negative rods 66, 67, 70
Differentiation of Peptostrecocci / *Micromonas micros* / *Finegoldia magna* 19, 86, 105
Differentiation of lecithinase positive *Clostridium* spp. 18
Differentiation of swarming *Clostridium* spp. 18
Differentiation of aerotolerant clostridia 70
Differentiation of *Clostridium difficile* 18
Differentiation of Clostridia-producing neurotoxins 52
Differentiation of Clostridia-producing large cytotoxins 19
Differentiation inside the *Clostridium clostridioforme* group 64
Differentiation of *Propionibacterium* spp. 77, 89, 111
Differentiation of *Capnocytophaga* spp. 23, 77

19) NOCARDIA

Identification of clinically most common *Nocardia* spp. 14
Identification of *Nocardia* spp. by antibiogram 14

20) YEAST

Differentiation of the most current *Candida* spp. 64
Identification of *Candida albicans* (differentiation of *C. dublinensis*) 19, 64
Rapid identification of *Candida glabrata* 108
Differentiation of *C. albicans* from *C. dublinensis* 73

© Copyright Rosco Diagnostica A/S

6 Alphabetic INDEX of Abbreviations and Codes

A) ACM = Acetamide Hydrolysis Diatabs (55721)
ADH = Arginine Dihydrolase Diatabs (56211)
ADO = Adonitol Diatabs (52011)
AER = Aerotolerant strains
ALA (dALA) = Porphyrin (d-Ala) Diatabs (57321)
Alk P = Alkaline Phosphatase Diatabs (55921)
ARA = l-Arabinose Diatabs (52121)
ANAgr = Anaerobic growth
AMP (AMP33) = Ampicillin 33 µg Neo-Sensitabs (70412)
AmpC = AmpC beta-lactamases

B) BACIT = Bacitracin 40 U Neo-Sensitabs (70812)
BaciLow (BaL) = Bacitracin Low 0.4U Diatabs (40211)
BE = Bile Esculin Diatabs (40411)
BrG = Brilliant green 100 µg Diatabs (40511)
BOR (BORON) = Boronic Acid 250 µg Diatabs (10041)

C) C-390 = C-390 40 µg Diatabs (41611)
CAT = Catalase
CEL = Cellobiose Diatabs (Non-stock)
CCFA = CCFA medium (Clostridium difficile)
CIT = Citrate Diatabs (56511)
CL500 = Cloxacillin 500 µg Diatabs (10031)
CLTN (CLOTN) = Cephalothin 66 µg (Cephalosporins) Neo-Sensitabs (72912)
COL (Co.10) = Colistin 10 µg Diatabs (41811)
Col dry adh = Colonies dry adherent
CYC = Cycloheximide Diatabs (58911)

D) DEF (DEFRX) = Deferoxamine 250 µg Diatabs (59611)
DUL = Dulcitol Diatabs (Non-stock)

E) ESBL = Extended spectrum betal-lactamases
ESC = Esculin Hydrolysis Diatabs (56611)

F) Fosfo (FOSFO) = Fosfomycin 70 µg (Fosfomycin+Glucose-6-Phosphat) Neo-Sensitabs (74212)
FRU = Fructose Diatabs (Non-stock)
α-FUC = Alpha-Fucosidase Diatabs (50111)
ß-FUC = Beta-Fucosidase Diatabs (59921)
Fura (FURAZ) = Furazolidone 50 µg Neo-Sensitabs (74412)

G) GAL = Galactose Diatabs (Non-stock)
α-GAL = Alpha-Galactosidase Diatabs (50211)
Gel (GEL) = Gelatine hydrolysis
Genta 250 (GN250) = Gentamicin 250 µg Neo-Sensitabs (43012)
GLU = Glucose Diatabs (52611)
α-GLU = Alpha-Glucosidase Diatabs (50411)
ß-GLU = Beta-Glucosidase Diatabs (50511)
γ-GLU = Gamma-Glutamyl Aminopeptidase Diatabs (46711)

H) HCF = Human clumping factor
HIP = Hippurate Hydrolysis Diatabs (56711)
HLR = High Level Resistance

© Copyright Rosco Diagnostica A/S

I)
IMP (IMIPM) = Imipenem 15 µg Neo-Sensitabs (74612)
IAC = Indoxyl Acetate Diatabs (59551)
IND (IN) = Indole Diatabs (Non-stock)
INO = Inositol Diatabs (Non-stock)
INU = Inulin Diatabs (52711)

K)
Kana 500 (KA500) = Kanamycin 500 µg Neo-Sensitabs (43112)

L)
LAC = Lactose Diatabs (52811)
LAP = Leucine Aminopeptidase Diatabs (46811)
LDC = Lysine Decarboxylase (LDC) Diatabs (56811)
LEC = Lecithinase
LIP = Lipase
LDC/IND = LDC/Indole (Lysine decarboxylase/Indole) Diatabs (58411)

M)
MAL = Maltose Diatabs (52911)
MALON = Malonate
MAN = Mannitol Diatabs (53011)
α-MAN = Alpha Mannosidase Diatabs (50711)
MBL = Metallo-beta-lactamases
McConk. = Growth in McConkey Agar
MGP = Methyl-α-D-glucopyranoside
MEL = Melibiose Diatabs (53211)
MTR50 = Metronidazole 50 µg Diatabs (43611)
MTR.5 = Metronidazole 5 µg Diatabs (59711)
MOT = Motility
MR = Methyl Red
MRS = Man, Sharp, Rogosa broth.
MSE = Mannose Diatabs (53111)
MTM = Growth on modified Thayer-Martin medium
Mupi (MUPIR) = Mupirocin 10 µg Neo-Sensitabs (75712)

N)
NA35 = Growth on nutrient agar at 35 °C
NAG (ß-NAG) = Beta-N-Acetylglucosaminidase Diatabs (50021)
NAL (NALID) = Nalidixan 130 µg Neo-Sensitabs (75812)
NO_3 = Nitrate Reduction Diatabs (43711)
Novo (Novo-5) (NOVO5) = Novobiocin 5 µg Neo-Sensitabs (76312)
NVS =Nutritionally variant streptococci

O)
O/129 = O/129 (Vibriostaticum) 150 µg Diatabs (45411)
ODC = Ornithine Decarboxylase (ODC) Diatabs (57011)
ODC/IND = ODC/Indole Diatabs (59121)
ONPG = ONPG (Beta-Galactosidase) Diatabs (50311)
OPT = Optochin 10 µg Diatabs (44211)
OXG = Oxgall 1000 µg Diatabs (44311)
OXI = Oxidase Diatabs (45711)

P)
PGUA (PGA) = Beta-Glucuronidase Diatabs (50611)
PGUA/IND = PGUA/Indole (Beta-Glucuronidase/Indole) Diatabs (59011)
PIGM = Pigment production
Poly (CO150) = Polymyxins 150 µg Neo-Sensitabs (77512)
PRO = Proline Aminopeptidase Diatabs (46911)
PSAER (PsS) = Ps. aeruginosa Screen 80 µg Diatabs (59311)
PYR = Pyrrolidonyl Aminopeptidase (PYR) Diatabs (47011)
PZA = Pyrazinamidase Diatabs (59811)

R)
R = Resistant
R^S = Most strains resistant
RAF = Raffinose Diatabs (53311)
RHAM = Rhamnose Diatabs ((Non-stock)
RIB = Ribose Diatabs (Non-stock)
RIFA (Rifa) (RIFAM) = Rifampicin 30 µg Neo-Sensitabs (77712)
RM = Rapid motility (4 hours at 35 °C)

© Copyright Rosco Diagnostica A/S

S) S = Sensitive (susceptible)
S^R = Most strains sensitive
SAL = Salicin Diatabs (Non-stock)
SFT = Sugar fermentation tests
SOR (SORB) = Sorbitol Diatabs (53711)
SPS = S.P.S. 1000 µg Diatabs (44611)
SUC = Sucrose Diatabs (53811)
SUP = Superoxol (30 % H_2O_2)
Strep 500 (ST500) = Streptomycin 500 µg Neo-Sensitabs (44712)

T) TDA or IND = TDA or Indole (Tryptophan Deaminase or Indole) Diatabs (57821)
TEL = Tellur 500 µg Diatabs (45011)
TRE = Trehalose Diatabs (53911)
TRIB = Tributyrin Diatabs (48821)
TRYP = Trypsin Diatabs (47211)
TTR = Tetrathionate Reductase Diatabs (57421)

U) URE (UR) = Urease Diatabs (57511)
URE/IND = Urease/Indole Diatabs (57611)
URE/TDA = Urease/TDA (Urease/Tryptophan Deaminase) Diatabs (57911)

V) V = Variable
Vanco (Van.5) = Vancomycin 5 µg Neo-Sensitabs (79312)
VP = Voges-Proskauer Diatabs (57711)
wk = weak

X) XYL = Xylose Diatabs (54021)
ß-XYL = Beta-Xylosidase Diatabs (50811)

$+^R$	=	rapidly positive
+	=	More than 90 % strains positive
$+^0$	=	75 - 90 % strains positive
V	=	26 - 74 % strains positive
0^+	=	10 - 25 % strains positive
0	=	Less than 10 % strains positive

If a number is written in the table, it refers to the percentage of positive strains.

© Copyright Rosco Diagnostica A/S